(KAM IX : 695)

MEMOIR 127 APRIL, 1930

CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION

THE BACTERIAL DISEASES OF THE BEAN

A COMPARATIVE STUDY

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IMPERIAL BUREAU
10 JULY 1930
OF MYCOLOGY

ITHACA NEW YORK
PUBLISHED BY THE UNIVERSITY
Received for publication November 25, 1929

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DISEASED STEMS AND PETIOLES OF A RED KIDNEY BEAN PLANT
These lesions are caused by *Phytomonas phaseoli*

THE BACTERIAL DISEASES OF THE BEAN

A COMPARATIVE STUDY

WALTER H. BURKHOLDER

The purpose of the investigation here reported was to separate and to distinguish, among the various diseases of the bean (*Phaseolus vulgaris* L.), those that are of bacterial origin. A number of such diseases occur on this crop, with symptoms for the most part similar and in some cases apparently indistinguishable from one another. The questions naturally arise as to how many of these diseases there are, which ones are causing economic losses to the crop, and how they may be recognized one from another. A knowledge of these phases of the subject is essential for the investigator before further work can be done effectively on any one of the diseases, since he must be able to recognize the disease with which he is working and to eliminate or be aware of the remaining bacterial diseases in his field of experimentation.

The bacterial disease of the bean caused by *Phytomonas phaseoli* has been recognized for a considerable period of time, but the diseases caused by *Phyt. flaccumfaciens* and *Phyt. medicaginis* var. *phaseolicola* have been known only during the past four or five years. Since these last-named diseases have been described, no comparative study has been made of the three; and, because they are so similar in many respects, confusion has arisen as to their distinctiveness and as to their identity. It was hoped in undertaking this investigation to clear up some of this confusion. In the progress of the work, however, it soon was found that still other bacterial diseases of this crop exist which further complicate the problem. Six distinct diseases have been recognized in this investigation, and in certain of these the pathogene appears to be composed of several different races. Consequently the entire problem of the bacterial diseases of the bean is more complicated than formerly it was thought to be.

In separating the various diseases and distinguishing them one from another, the work naturally falls into three parts: first, the symptomatology of the diseases on the bean; secondly, the host range of the pathogenes; and thirdly, the etiology, a study of the morphology and the cultural characteristics of the pathogenes. Other phases of the problem are numerous and important, but they are not phases that would facilitate the separation of these diseases which are under consideration. In presenting each disease separately in this memoir, the above three divisions of the problem have been dealt with mainly. What is known concerning the life history of the organism, however, has not been neglected. In treating this problem in such a manner it has seemed justifiable to duplicate certain of the previous

work done with these diseases. All cultural characteristics of the pathogenes have been determined under identical conditions (and on the same media), which is an essential precaution in showing similarities and distinct differences. The symptomatology of the various diseases on the bean host has been studied under similar conditions, and the host range for each pathogene determined in a like manner. By following such a procedure it is felt that a certain amount of error has been eliminated which might exist in separate investigations conducted in separate laboratories.

So far as is known, only one important bacterial pathogene of the common bean has been neglected. *Phyt. phaseoli* var. *sojense*, while found primarily on the soybean, does produce an infection on the common, or garden, bean, and it is regretted that this organism was not included in the comparative study. Wolf (1924) and Miss Hedges (1924) have reported their investigations on the soybean organism, however, and find that culturally it agrees with *Phyt. phaseoli*, except for a few minor exceptions. The problem appears to be primarily one of pathogenicity. Several other bacterial pathogenes have been reported as being pathogenic to the bean, but they are ones which are of little importance to this host. *Phyt. solanaceara*, *Phyt. tobaca*, and *Phyt. aptata* might be mentioned in this class.

METHODS

In any comparative study, the methods used for conducting the experiments are of primary importance. In the following paragraphs are set forth the methods employed in making inoculation experiments and in determining or describing the pathogene in culture. Wherever a deviation from these methods is made, it is mentioned in the text.

INOCULATION EXPERIMENTS

For conducting inoculation experiments, plants were grown in a greenhouse at a relatively high temperature. The common bean (*Phaseolus vulgaris*) and many of its related species thrive best in a warm atmosphere, a vigorous succulent growth is obtained, and furthermore the plants are not so subject to damping-off as they are at a lower temperature. The clovers and the garden peas are an exception to this rule, and these plants were grown in a cooler house. The greenhouse used for the inoculation experiments on beans had a constant-temperature-control apparatus which held the house at approximately 78° to 80° F., except during bright, sunny days when the temperature would rise to 90° F., or even more, after the steam had been shut off. Under these temperature conditions, infection developed much more rapidly when plants were inoculated with such organisms as *Phytomonas phaseoli* and *Phyt. flaccumfaciens* than at lower temperatures, such as 60° to 65° F. With some of the other pathogenes, as good results were obtained at these lower temperatures but, from observation, infection was in no respect better. *Phyt. medicaginis* var. *phaseolicola* appears to spread

in the field more rapidly and do more damage in a cool season than in a warm season. In the greenhouse, however, excellent infection is obtained at 80° F.

What part humidity plays is still in doubt. When inoculations were made by injuring the plants, no extra care was taken to place them in a moist atmosphere. Such inoculated plants were allowed to remain on the greenhouse benches under the usual conditions, and inoculations were made at any time during the day. When plants were inoculated by spraying with a water suspension of the organism, without injuring them in any manner, it was considered wise to place them in a moist chamber for about twenty-four hours. This procedure, however, was not based on any experimental evidence.

The technic of inoculation by injuring the plants was as follows: The pathogene was grown on an agar slant at 27° C., and its age when inoculations were made was, as a rule, from twenty-four to forty-eight hours. Seedlings were used which had advanced beyond the primary leaf stage but were still tender and succulent. A small part of the bacterial mass was placed on a very sharp pointed scalpel and the plant was stabbed with this contaminated instrument. The injury was made in most cases at the primary leaf node and extended into the pith. When pods were used in the experiments they were inoculated in a similar manner, the injury being made either on the suture or on the side.

When experiments were conducted without injuring, the inoculations were made by spraying the plants with a water suspension of the pathogene from the agar slants. The bacteria were washed from the agar with tap water and sprayed from an atomizer. The plants were then placed in a moist chamber for approximately twenty-four hours. When pods were used, the water suspension not infrequently was painted on with a camel's-hair brush.

Some confusion exists in the literature as to the correct scientific name to be applied to certain of the leguminous plants referred to in this bulletin. Bailey's *Manual of Cultivated Plants* has been followed here in so far as specific names are concerned. Where varieties have been established on the character of determinate or undeterminate growth habit, they have been disregarded.

PURE-CULTURE STUDIES

Before the cultural studies were begun, all strains of the various species and varieties of bacteria used were inoculated into bean plants. After infection had taken place and the disease had progressed to a considerable extent, the organism was reisolated from diseased tissue as far removed from the point of inoculation as possible. Dilution plates were then made and a single colony was transferred to a tube. When the pathogenicity of this culture had been proved, the cultural work was begun. It should be stated here that prior to the foregoing procedure an attempt was made

to rejuvenate all strains of pathogenes which had been in pure culture for a year or more. This was done by growing them for several months in their natural habitat, that is, as pathogenes on the bean plant. It is felt by the writer that in comparative studies, especially with fungi, many of the so-called physiologic species are brought about merely by the various lengths of time during which the organism was grown in pure culture. The writer has shown that this is the case with the fungus *Fusarium martii* f. sp. *phaseoli*, and that a return to the natural habitat will cause a return to the original morphologic and physiologic state (Burkholder, 1924). It was hoped that this would hold true also with the bacterial pathogenes.

In the preparation of media, stains, and other materials, and for their method of use, the *Manual of Methods for Pure Culture Study of Bacteria*, issued by the Society of American Bacteriologists, was followed unless otherwise stated. The formulae for preparing Uschinsky's solution, Fermi's solution, and Cohn's solution, were obtained from Smith's *Bacteria in Relation to Plant Diseases*. Andrade's indicator was used in all the sugar broths. To determine cellulose digestion, a broth containing 0.2 per cent of peptone and 0.1 per cent of K_2HPO_4 was used, in which was placed a narrow strip of filter paper extending above the medium. In determining the color of pigment production of an organism in or on any of the media, Ridgway's *Color Standards and Color Nomenclature* was employed.

Variations occurred in the same strain in different lots of media made according to the same formula but with ingredients from different sources. While this was true to a certain extent with beef-extract agar, it was very evident at times with gelatin. The most striking cases are recorded later in this memoir.

In studying the bacteria, all cultures, with the exception of gelatin, were kept in an incubator at a constant temperature of 27° C. Gelatin cultures were incubated at 21° C. The 27°-C. temperature was decided on through the observation that all cultures thrived well at that temperature. Some of the organisms, however, appeared to grow equally well at a somewhat lower temperature.

In measuring individual cells the Congo red negative stain was used as described by Benians (1916). It was felt that for the careful study of morphology, and especially of size, results obtained by the direct staining methods are less nearly accurate.

THE BACTERIAL BLIGHT OF THE BEAN CAUSED BY PHYTOMONAS PHASEOLI

Although a bacterial disease of beans was reported as early as 1892 both by Beach (1893) and by Halsted (1893), Smith (1898) was the first to isolate a bacterium from the bean, prove its pathogenicity, and give the organism a name. First described as *Bacillus phaseoli*, references to the pathogene may be found in literature under the following generic names

in accordance with the classification which the various writers prefer: Pseudomonas (Migula), Bacterium (Lehman and Neuman, and E. F. Smith), and Phytomonas (Bergey). In this memoir the Bergey classification is adopted.

For approximately twenty-five years after the discovery of *Phytomonas phaseoli*, all references to a bacterial disease of beans assumed this organism to be the etiological agent. Possibly in the great majority of the cases this assumption was true, and the other bacteria pathogenic to this host are, no doubt, of recent appearance. One reason for believing this, is the fact that only in recent years in the United States have the bacterial blights been so severe. It seems inconceivable that *Phyt. flaccumfaciens* or *Phyt. medicaginis* var. *phaseolicola*, two organisms which are much more destructive at times than *Phyt. phaseoli*, would have been overlooked if they were present on the bean, or that their virulence should have increased in later years. Furthermore, these various pathogenes are not limited to *Phaseolus vulgaris*, and it is entirely possible that they appeared originally on other species and that their appearance on the bean is of but recent occurrence. Pathogenic on a wild species or slightly pathogenic on some cultivated host, they very easily could have been overlooked. After they once got a foothold on the bean plant, and especially the seed stock, they spread rapidly over the country.

Since *Phyt. phaseoli* is the oldest and the best-known species of the bacteria attacking the bean, it is the one with which all the others are compared. For this reason this pathogene and the disease which it produces are treated first.

LIFE HISTORY

In order to recognize and understand certain symptoms of the disease caused by *Phytomonas phaseoli* and to differentiate them from the symptoms of the other bacterial diseases of the bean, a knowledge of certain phases of the life history of the pathogene is helpful. The term *life history* is used here in a broad sense, to designate not only the methods of overwintering of the organism, and the methods of infection, but also the tissue of the plant that is attacked and the peculiar invasive ability of the organism in the host. While these features are stressed in the following sections, what is known of the entire life history of the pathogene is also reported.

One of the most important stages in the life history of an organism, from the standpoint of primary infection and of control, is the stage in which it passes the winter. The usual way in which *Phyt. phaseoli* survives the cold months is in a dry condition in or on the bean seed. Here it is in a highly resistant state, and under laboratory conditions at Ithaca, New York, it has been known to remain viable for a period of three years. Rapp (1919), in Oklahoma, is of the opinion that the pathogene in this state will die within a year's time, and Sackett (1926), in Colorado, reports

success in reducing the amount of disease by planting two-years-old seed. Temperature, moisture, and the manner of storing the seed, no doubt play an important part in such viability tests.

Muncie (1917 b) and recently Patel (1929) have shown that *Phyt. phaseoli* can overwinter also in the soil. One experiment has been conducted by the writer on this phase of the subject, and, while not conclusive, is reported here merely for what it is worth. This experiment differs from those of Muncie and Patel in that it was conducted in the field, and, although having a more practical bearing, it lacked the niceties of the laboratory experiments. A plot 100 feet by 50 feet in size was planted to beans, and, when germination had taken place and the seedling stage had passed, the plants were inoculated heavily with the pathogene. All plants became severely infected, and in the fall were not pulled and removed as is usually the case in the sections growing dry shell beans. Thus the soil was much more severely contaminated with the organism than is usual. *Phyt. flaccumfaciens* probably was present, besides *Phyt. phaseoli*. In the following spring the plot was plowed and again sown to beans. On either side, plots of similar size were used as checks. In August, when counts were made of the number of blighted plants in each plot, it was found that the checks had slightly more diseased individuals than the treated plots had. Continuous observations before this time had shown that the disease had not originated in the treated plot and spread to the checks, but had appeared simultaneously over the entire planted area. The disease had come in on the seed, or had been brought in, in some other manner, from the outside. This is merely a single experiment, and such conditions as the amount of organic material in the soil, hydrogen-ion concentration, and other related factors, might alter the case.

The fact that the bacteria in the soil can survive a winter under New York conditions is probably of no great importance. Rotation of crops is practiced, and even in the western counties, where beans are grown as a major crop, the rotation is seldom less than three years. Can the organism survive for a period of that length? From observation, probably not, since the disease is of no economic importance when seed from disease-free fields is used for planting.

Under New York conditions, infected seed introduce the pathogene into a field in approximately 100 per cent of the cases. The life history of the pathogene in relation to its host plant, then, may proceed in the following manner: With the germination of the planted seed, the bacteria become active, and their number in the cotyledons determines the severity of early infection, weather conditions being favorable. With severely infected seed the growing tip may be destroyed by the time the plant is above ground, giving rise to the so-called *snakeheads* and frequently causing the death of the seedling. This consequently reduces the chances of the particular pathogene's continuing and infecting other host plants. In

less severely infected seed, or when weather conditions are less favorable for the bacteria, they may be confined at first entirely within the cotyledons. Here they may multiply in the fleshy tissue, and be washed or splashed by rain to various parts of the young growing plant. An injury is not necessary for the bacteria to cause infection, and almost any tissue above ground may be invaded. In some cases, during the early stages of development the pathogene in the cotyledons may enter the stem of the plant through the vascular system and establish itself in the xylem vessels. The walls of these vessels apparently are very resistant to the organism, since the bacteria may exist there for weeks without breaking through into other tissue. In the xylem vessels, however, the bacteria may travel throughout the plant. Their progress usually is upward, probably owing to the water stream; but they may also travel downward, and in rare cases have been recovered from the roots of diseased plants. When they are confined to the stem alone, little effect may be produced on the plant aside from a slight stunting; but when they pass into the leaves, the young growing tips, and other plant parts, wilts and necrotic lesions may result. In their passing through the xylem vessels of the pedicel into fairly mature pods and infecting the seed, no external evidence of the presence of the bacteria may be noticed. Zaumeyer (1929) has recently described in detail the tissues involved in this latter process.

The tendency for *Phyt. phaseoli* to systemically invade a plant was first noted by Barlow (1904) and was later emphasized by the writer (Burkholder, 1921). Plants so infected are not the rule in the fields of New York; nevertheless they do serve a purpose in the life history of the organism in this climate. The first month-and-a-half of the growing period of the bean plant is unfavorable for the development of the blight. June and early July are inclined to be cool, and *Phyt. phaseoli* prefers a warm temperature. In late July and during August, when the pathogene has come to the surface in systemically infected plants through various lesions, these plants act as a source of inoculum for the spread of the disease. At times, in a bean field under close observation, one may watch the spread of the blight in an ever-growing radius about such a plant. The overwhelming majority of the leaf and pod lesions which one observes in a field, are local and of exogenous origin. Even in these lesions, however, and especially on the leaves, the pathogene usually localizes or develops more rapidly in the xylem vessels.

The rapid development of the blight in a field depends considerably upon warm weather. It may be observed that the disease is more prevalent in hot seasons than in cool ones. In the greenhouse, infection is more easily obtained at 80° F. than at 65° F., and at the latter temperature the leaf spots develop very slowly. One observation in the field on the relation of temperature to the blight is possibly worthy of recording here. In 1922 the writer had a small field of beans at Ithaca on a northern slope.

Twice these beans were inoculated by spraying with a water suspension of *Phyt. phaseoli*, but the disease progressed very slowly. On a southern slope in the same field, and only 300 feet distant, a variety test of beans was being conducted. In this plot the blight broke out and spread rapidly from plant to plant, causing considerable damage. The difference in temperature in the two plots of beans was held accountable for the difference in the severity of infection in the two places. The pathogene progresses more rapidly in the plant tissue at high temperature than at low.

With the infection of the seeds of diseased plants, the life history of *Phyt. phaseoli* is completed. The organism gains entrance to the seed by passage through the vascular system or through local lesions on the pod. Seeds that are but slightly infected are more likely to introduce the organism into the field in the following season than are severely infected seeds. The latter are usually removed during the process of cleaning and picking in the seed houses, or, if planted, they may decay.

SYMPTOMS

General

Generalized symptoms may occur on the bean plant when the vascular system of the stem has become invaded by the pathogene. The severity of the symptoms varies with the proportion of the vascular system that is invaded. In light infections a stunting of the plant often will occur and will scarcely be noticeable except in comparison with normal individuals. On such lightly infected plants, an incipient wilting may take place during hot, dry days, but a recovery is always made in the evenings.

When the bacteria begin to invade the entire system a true wilt usually results. Seedling wilts are not infrequent, and at times this symptom arises very quickly. In older plants a leaf, a branch, or the entire plant may wilt (Plate V, D and E), and the progress of the wilt is usually in this order.

When seeds which are fairly severely infected, but not sufficiently so to injure the germination, are planted, a seedling may be killed as it emerges from the ground, or the bacteria may attack the growing tip of the plant and destroy it, leaving only the cotyledons. The appearance of such seedlings has given rise to the name *snakehead*. It should be stated here, however, that certain insects, fungi, bacteria, and seeds broken by threshers may cause a similar condition.

On the leaf

In the most typical leaf spots of beans due to *Phytomonas phaseoli*, shown in figure 1, the lesions may first appear as minute water-soaked spots or light green wilted areas, which, as they enlarge, dry out and become brown and brittle. About the edges of such lesions is a yellow

FIGURE 1. LEAF OF RED KIDNEY BEAN SHOWING LESIONS CAUSED BY PHYTOMONAS PHASEOLI

border, and surrounding this there is often a narrow pale green zone. One or more of these spots may increase in size and involve a large part of the entire leaflet. After a heavy rain or windstorm, such leaves have a very ragged and torn appearance. These typical lesions are, as a rule, due to external infection, but at times they may develop from the bacteria rising from the vascular system.

Several other types of leaf spots of beans have been observed and have been described before. One of these occurs only on young leaves. In this type the leaf is covered with small, angular spots which are at first water-soaked and dark green in appearance, but which later dry out and become brown. The necrotic tissue in the lesions is in the islets between the veins, and infection of the vascular bundles does not appear to have taken place.

On several occasions an anomalous type of lesion on the bean leaf has been observed. The varieties affected were the Red Kidney and an unknown green-pod variety. The spots in each case varied somewhat in size, but were not large, and were slightly raised above the healthy surface of the leaf (Plate II). In color they were a red-brown, and not unlike the leaf spot of lima beans due to *Phyt. vignae*. Isolations were made from a number of such lesions, and in all cases the pathogene appeared to be *Phyt. phaseoli*. No careful study of the cultures was made, however, outside of determining their pathogenicity on the common bean. Whether or not they were a distinct variety or race of *Phyt. phaseoli* is not known.

On the stem

Phyt. phaseoli produces water-soaked lesions on the stems of bean seedlings, as may be observed where diseased seeds have been planted. As these lesions grow older, they lose their water-soaked appearance, dry down, and become reddish brown in color. On older plants the water-soaked condition seldom is evident. Stem lesions usually are in the shape of streaks and are always longitudinal with the stem.

A second type of lesion found on the stem of the bean and due to this organism is the stem girdle. This injury sometimes causes considerable damage to the crop. The lesion is produced at the cotyledon, or more frequently at the first leaf node, or less frequently higher up on the plant. In these cases the vascular system is infected and the bacteria come to the surface in the leaf traces, resulting in the girdle. The color of these lesions is reddish brown, as is that of the streaks on the stem. Plants affected with the stem girdle become weakened at the infected node and are easily toppled over during a windstorm.

On the pods

Several types of lesions occur on bean pods, as may be seen in Plates II and IV and in figure 11 (page 46). The number of infections, the tender-

ness of the pod, and certain environmental factors, no doubt contribute in producing the differences in size and shape of the spots. All lesions are at first water-soaked and dark green in color, but later the tissues may become dry, sunken, and brick red. The process of drying begins at the outer edge of the spot and extends inward, and it is not uncommon to find a green, water-soaked lesion with a reddish, dry border. In many cases a yellowish crustation, due to the bacteria oozing out and drying over the lesion, covers the spot.

When the bacteria enter the pod by the vascular system, they may produce a long, irregular lesion along the suture (Plate IV, B). In other respects, however, the lesions so produced resemble those described above which arise from an external infection.

On the seeds

On white seeds, *Phyt. phaseoli* produces yellow spots of various sizes. When an entire seed is involved, it may be wrinkled and yellow, and appear as if it had been varnished. On colored seeds, small lesions are impossible of detection, and larger ones cannot be distinguished from anthracnose spots or abnormalities due to frost injury. Likewise, when immature seeds are harvested they may show a slight wrinkling and discoloration which at times could be mistaken for a bacterial infection.

If the bacteria enter the seed through the vascular system, and infection is light, they may produce only a yellow spot at the hilum, but in more severe cases they spread out underneath the seed coat. Seed slightly infected, either at the hilum or on the side, may escape detection during picking in the seed house and be used the following year for planting.

INOCULATION EXPERIMENTS

On the bean

Many of the symptoms just described have been reproduced on plants in the greenhouse by artificial inoculation, especially the symptoms connected with systemically infected plants. Red Kidney was the variety used, and the temperature maintained was approximately 80° F. More success in producing a vascular infection resulted from employing recently isolated strains of the pathogene than with strains that had been in culture for a year or more. Plants that were inoculated by pricking at the cotyledon node and were then placed in a temperature of 65° F. did not wilt as did those shown in Plate V, D and E, which were at 80° F. The plants in the lower temperatures, however, developed typical leaf spots on the lower leaves, and the bacteria in such lesions could be traced down the xylem vessels to the point of inoculation on the stem. When plants were inoculated through injuries, positive results were obtained in 100 per cent of the cases. When plants were sprayed with a water suspension

FIGURE 2. WILT OF BEAN SEEDLING CAUSED BY PHYTOMONAS PHASEOLI

The seedling was artificially inoculated at the cotyledon node

of the bacteria, and no mechanical injury was made in the plants, the results were variable. Environmental conditions favorable for infection under such circumstances are not yet sufficiently understood.

Cross inoculations

Inoculation experiments were conducted on a number of legumes which are listed in table 3 (page 77). On the sieva bean (*Phaseolus lunatus* L.), the scarlet runner bean (*P. coccineus* L.), and the hyacinth bean (*Dolichos lablab* L.), the symptoms produced by the disease were very similar to those on the common bean (*P. vulgaris* L.). Good infection was obtained also on the white-flowering lupine (*Lupinus polyphyllus* Lindl.). With this species, inoculations were made by pricking the crown and introducing the organism. A wilt of the leaves and the petioles resulted, and the pathogene was easily recovered from the leaves. Gardner (1924) reported *Strophostyles helvola* (L.) Britton as a host of *Phytomonas phaseoli*, but this species was not used in these experiments.

In 1924 the writer reported some inoculation experiments on various species of legumes grown in the garden with some varietal susceptibility tests of *P. vulgaris* (Burkholder, 1924). In the light of the present series of carefully conducted experiments in the greenhouse, the former inocula-

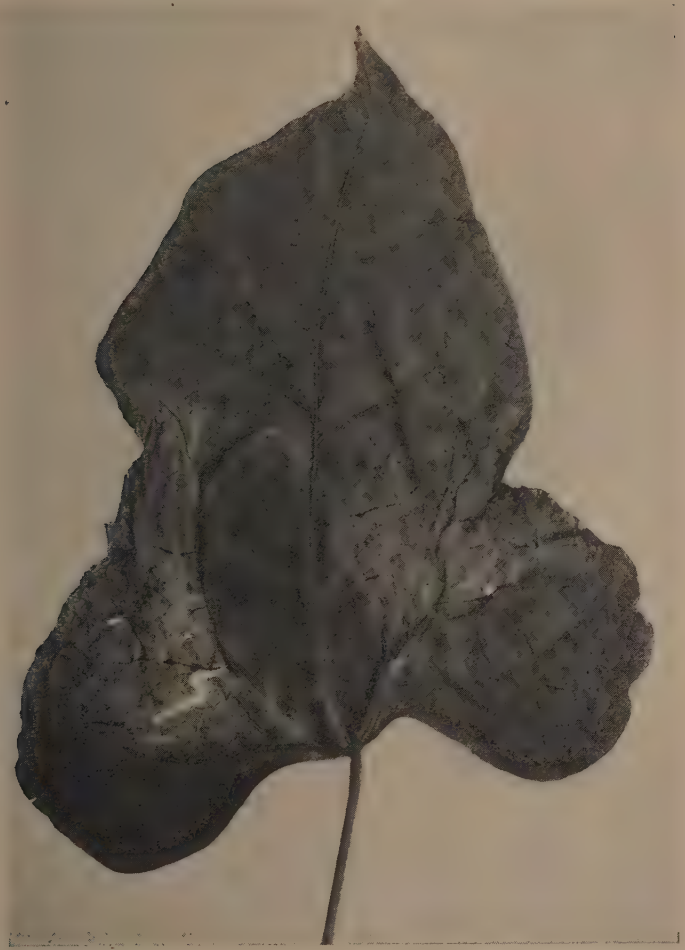

FIGURE 3. PRIMARY LEAF OF A BEAN PLANT SHOWING LESIONS RESULTING FROM INOCULATING THE STEM OF THE PLANT WITH PHYTOMONAS PHASEOLI

tion tests do not appear in all cases to be trustworthy. In these latter experiments, negative results always were obtained on the following species: adzuki bean (*P. angularis* Wight.), cowpea (*Vigna sinensis* Endl.), yard-

long bean (*V. sesquipedalis* W. F. Wight.), soybean (*Glycine max* Merr.), kudzu vine (*Pueraria hirsuta* Schneid.), broad bean (*Vicia faba* L.), field pea (*Pisum sativum* L. var. *arvense* Poir.), sweet clover (*Melilotus alba* Desr.), and alfalfa (*Medicago sativa* L.).

ETIOLOGY

Many strains of *Phytomonas phaseoli* have been isolated, from time to time, from various types of symptoms and from various parts of the bean plant (*Phaseolus vulgaris*). Most of these strains have been from specimens gathered in New York, but in two cases they have been from foreign countries — one from Bermuda and one from Switzerland. Besides being isolated from *P. vulgaris*, cultures have been obtained from *P. lunatus* and *P. coccineus*. In all these instances the strains have been practically identical, which indicates that *Phyt. phaseoli* is a very stable species.

In culture the organism does change somewhat. While it still may produce as vigorous a growth on agar, certain biochemical reactions in culture appear to diminish though they have never been observed to be entirely lost. Strains that have been grown in culture for a number of years will not liquefy gelatin as rapidly as will recently isolated strains, and, similarly, the action on milk is delayed. Consequently, in comparing old and young cultures there frequently are shown minor differences which would not exist if the strain had been under identical conditions previously.

Morphology

Phyt. phaseoli is a medium-sized rod with rounded ends, occurring singly or in pairs. Filaments have never been observed in this species. Often certain cells appear to be oval, but in cultures twenty-four hours old the variation about the mode is not great. Such cultures, on beef-extract agar (pH 6.9) and incubated at 27° C., show the following dimensions: 1.9μ (1.35 to 3.6μ) by 0.87μ (0.45 to 1.35μ).

The organism is motile by means of a single polar flagellum. It is Gram-negative, not acid-fast, and capsule production has not been demonstrated. When grown on a medium containing dextrose a large amount of gum is produced. The pathogene appears to be a fairly strict aerobe.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated)

Beef-extract agar slants (pH 7.0). A moderate growth develops along the streak within twenty-four hours. Growth becomes abundant, filiform, wet, glistening, and amber yellow in color. The consistency is often very watery. From appearance the cultures are not amorphous, since various shades and intensities of the pigment occur within the bacterial growth.

Beef-infusion agar slants (pH 7.0). Growth does not vary to any appreciable extent from that on beef-extract agar. Considerable difficulty has been experienced, however, in keeping this species alive on this agar. It often dies within a week's time. This inability to remain viable on beef-infusion agar was noticed with several different strains of the pathogene.

Beef-extract agar plates (pH 7.0). Seven-days-old colonies are circular, approximately 3 to 5 millimeters in diameter, glistening, and primulin

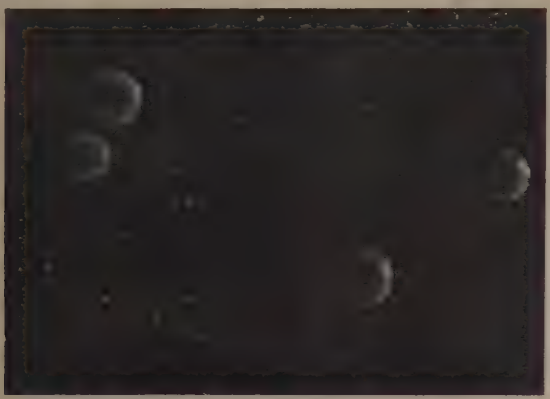

FIGURE 4. SEVEN-DAYS-OLD SURFACE AND DEEP COLONIES OF PHYTOMONAS PHASEOLI ON BEEF-EXTRACT AGAR (pH 7.0) AT 27° C. × 2

yellow in color. The consistency is watery to butyrous. The edges are entire, and their color is not so intense as that of the center of the colony.

Beef-extract bouillon (pH 7.0). The broth becomes cloudy in twenty-four hours, and very cloudy in forty-eight hours. A yellowish ring is formed, and later a heavy sediment.

Gelatin stab (pH 6.6). (Temperature 21° C.) Growth occurs along the stab within twenty-four hours. On the second day slight liquefaction may be observed, which proceeds slowly until it is complete in approximately five weeks. Various types of liquefaction occur at different times, with different strains, and in different lots of gelatins.

Milk. Growth is first observed in about four days, when the medium begins to clear very rapidly. A yellow surface layer is formed, and a more or less heavy sediment appears at the bottom. Within several weeks the upper half of the tube is a clear yellowish liquid, the lower part is cloudy, and a great many tyrosine crystals are formed in the digesting casein. The medium is rendered alkaline.

Litmus milk. Growth is very similar to that in plain milk, with the exception of the color reaction. Litmus is reduced, although there is always a trace of purple in the tube. At the end of six weeks the upper part is a slightly reddish liquid, while the lower part is a muddy yellowish brown medium with crystals.

Brom cresol purple milk. The medium soon becomes a rather clear reddish purple liquid with a muddy sediment. As with all milk cultures, tyrosine crystals are formed in abundance.

Uschinsky's solution. A very faint clouding occurs in about ten days. Within two weeks there is a fair cloudy growth, but no further change takes place.

Fermi's solution. As a rule there is no growth. In a few cases a light cloudy growth has been observed.

Cohn's solution. There is no growth.

Potato cylinders. In twenty-four hours there appears a very good watery growth along the streak, which is primrose yellow in color. The growth soon covers the entire surface, and the potato cylinders begin to dissolve into a rather slimy mass. This breaking-down of the potato is one of the striking peculiarities of this pathogene. The color of the mass soon becomes a reed yellow.

Cellulose digestion. A light cloudy growth appears in the broth in twenty-four hours. Within a week or two, a yellow color may be noticed on the filter paper just above the surface of the liquid and at the end of the paper an inch or so above the liquid. There is no digestion of the cellulose.

Starch agar plates (pH 6.6). Plates streaked five or six times, and tested with a saturated solution of iodine in 50-per-cent alcohol, show the starch to be practically all digested at the end of two days.

Dextrose broth. A cloudy growth occurs in twenty-four hours in the open arm of a fermentation tube. No acid nor gas is produced.

Saccharose broth. A cloudy growth occurs in twenty-four hours in the open arm of a fermentation tube. No acid nor gas is produced.

Lactose broth. A cloudy growth occurs in twenty-four hours in the open arm of a fermentation tube. No acid nor gas is produced.

Maltose broth. A cloudy growth occurs in twenty-four hours in the open arm of a fermentation tube. No acid nor gas is produced.

Indol production. The organism grows well in trytophane broth, and determinations made at the end of one and of four days with the Ehrlich-Böhme test show no indication of indol production.

Nitrate reduction. The pathogene does not reduce nitrates to nitrites. Tests made at the end of two, four, and eight days have all been negative. The method used was that of sulfanilic acid and a-naphthalamine in acetic acid.

Hydrogen-sulfide production. The pathogene does not grow on Wilson's medium. On lead-acetate agar there is a good growth along the stab

and on the surface of the agar. A very slight browning takes place along the stab. If hydrogen sulfide is produced, which is contrary to other reports, there is only a slight production.

Chromogenesis. The distinct yellow pigment appears to be produced on all types of media. In liquid media, yellow rings and yellow sediments are distinctly evident. The pigment is not water-soluble.

Oxygen relations. From its behavior in fermentation tubes and in shake agar cultures, the organism appears to be a rather strict aerobe.

Longevity. In a dry condition, on or in the seed of its host plant, the pathogene will remain viable for several years. In culture, however, the life of the organism is comparatively short. Transfers should be made at least once a month to prevent the strain from dying out.

Virulence. It has been stated in literature that the organism does not lose its pathogenicity or its virulence in pure culture. The reverse statement also has been made. In working with a great many strains, the writer has always been able to produce lesions on green pods no matter how long the strain had been in culture. In the endeavor to produce a seedling wilt or to cause a systemic infection of a bean plant, success appears always to be certain with a recently isolated strain, but negative results are often obtained with a strain that has been in culture for a year or two. Possibly this accounts for the difficulty obtained from time to time in producing an infection of the vascular system.

Thermal death-point. A few thermal-death-point tests have been made with this species, but with indefinite results. The thermal death-point appears to be low, probably around 48° C.

Brief description

Phytomonas phaseoli is a medium-sized rod with rounded ends, occurring singly or in pairs. The average length of the cells is 1.9 μ. The organism is motile by means of a single polar flagellum. It is Gram-negative, not acid-fast, and apparently a fairly strict aerobe. Capsules have not been observed.

On agar slants the growth is good, filiform, and yellow in color. The consistency of the culture is watery to butyrous; broth cultures are cloudy in twenty-four hours; gelatin is liquefied; milk is peptonized without a curd; there is no acid nor gas in dextrose, saccharose, lactose, or maltose broth; starch is digested; nitrates are not reduced to nitrites; indol is not formed; hydrogen sulfide is not produced or but slightly; there is no growth in Fermi's or in Cohn's solution; growth in Uschinsky's solution is slight; the thermal death-point is approximately 48° C.

The organism is pathogenic on the common bean (*Phaseolus vulgaris*) and on several closely related species.

A DISEASE OF THE BEAN CAUSED BY A VARIETY OF PHYTOMONAS PHASEOLI

(Var. *fuscans* n. var.)

In August of 1924, specimens of diseased bean leaves were collected in a garden at Montreux, Switzerland. The variety of bean was unknown, but it was of the green-pod type; and the lesions on the leaves of the plants were typical of those caused by *Phytomonas phaseoli*. The spots varied in size, were brown and necrotic, and were surrounded by a yellowish border. In December of the same year, attempts were made to isolate an organism from these specimens although the leaves were in a very dry condition. A part of a lesion was macerated in sterile water and dilution plates were made. In all the plates, yellow, rapidly growing bacterial colonies appeared, very much like *Phyt. phaseoli* in appearance except for one characteristic. The agar medium about all the colonies became, after a week's time, a distinct brown in color. It was thought at first that the organism produced a water-soluble brown pigment which dissolved throughout the neighboring medium. This interpretation, however, was found later to be incorrect. Further dilution plates were made with the pathogene to separate it from any contaminating organism; a single colony was transferred to a tube and its pathogenicity on the bean was proved. A reisolation then was made from one of the infected plants, and the culture was saved for further work.

In August, 1927, green-bean pods were obtained in a market at Lucerne, Switzerland, which showed dark green, water-soaked spots apparently due to a bacterial invasion. Later, from these spots an organism was isolated similar in appearance to the one just described. The colonies were yellow and the surrounding medium became a distinct brown in color. This second isolation of this bacterium appeared to be an indication that the pathogene was not uncommon in Switzerland, and from the nature of the lesions observed it caused a disease similar to the blight due to *Phyt. phaseoli*. The pathogenicity of this organism was tested by inoculating it into a bean plant, and after infection took place it was reisolated and saved for further work.

Miss Florence Hedges informed the writer that she also had isolated what appeared to be the same organism from diseased bean seed which had been imported from South America. The pathogene probably is widespread, but, owing to its similarity to *Phyt. phaseoli*, has been overlooked.

INOCULATION EXPERIMENTS

On the bean

Inoculation experiments were conducted on Wells' Red Kidney plants in a greenhouse with a temperature of approximately 80° F. While the organism never completely lost its pathogenicity in pure culture, its viru-

lence at times appeared to wane and reisolations were deemed necessary. During the progress of the work a few infected plants were continuously on hand in the greenhouse so that reisolations could readily be made. Inoculations on seedlings made by pricking the stem at the cotyledon node usually caused a wilting of the plant. The incubation period varied; as a rule it was about a week, but in some cases the time was longer. There were no peculiarities of this wilt of seedlings to distinguish it from such a symptom produced by any of the other xylem-infecting organisms. The seedling simply wilted, although, as a rule, rather slowly.

When young plants past the seedling stage were inoculated, certain characteristic symptoms did occur. Few of the plants died, though many do which are infected with *Phytomonas phaseoli*, *Phyt. flaccumfaciens*, or *Phyt. medicaginis* var. *phaseolicola*. A leaf or perhaps a tip of a branch or a young pod of the inoculated plant would wilt and die, but the plant as a whole would struggle on. At the point of inoculation, where the tissue of the stem was injured, a necrotic lesion would develop. This lesion extended slowly up the stem, and to a less degree downward. The lesions were reddish brown in color, and were dry; and cracks longitudinal with the stem would sometimes appear. In some cases there was a slight hypertrophy of the tissue about the injury. This symptom rather distinguishes this disease from others of its type.

Infection may be obtained on pods of the Red Kidney bean if a water suspension of the pathogene is sprayed or painted on them. If the pods are injured, infection takes place more readily and with greater certainty, although injury is not necessary. The first symptom of the lesion on the pod is a small water-soaked pustule, which, however, soon breaks down and a green water-soaked spot is formed. This condition persists for some time, but later the lesions dry out and become brick red and slightly sunken. When artificially inoculated pods are gathered in the greenhouse and stored in envelopes in the laboratory, the lesions remain green in appearance and rather gummy. In this respect it does not vary greatly from the spots produced by *Phyt. phaseoli*. The early formation of a pustule is the only difference. Inoculation of the pod at the peduncle has resulted in water-soaked lesions along the suture (Plate IV, C). These are indistinguishable from such lesions produced by *Phyt. flaccumfaciens*. It was thought that some of the lesions on the stem and the pods had a brownish coloration, which was lacking in symptoms due to the other bacteria. In other instances, however, this color symptom was entirely absent. Lesions produced on the leaves of Red Kidney plants in the greenhouse could not be distinguished from those produced by *Phyt. phaseoli*. They were in appearance similar to those found in Switzerland.

In a few cases, young plants inoculated with this pathogene and left in a greenhouse at a temperature of 65° F. did not develop the disease. Other plants, however, of the same lots showed unmistakable symptoms when placed in a temperature of 80° F. A high temperature seems necessary for the development of the disease.

Cross inoculations

Besides inoculation of the common bean (*Phaseolus vulgaris*), infection was obtained also on the sieva bean (*P. lunatus*) and the scarlet runner bean (*P. coccineus*). On the sieva bean, definite necrotic lesions on the leaves followed inoculation of the stem. This was more likely to be the result than a primary wilting. In these instances the bacteria could be traced up the xylem vessels in the stem to the lesions on the leaves. Water-soaked lesions were obtained also on the pods. On the scarlet runner bean, the lesions were, as a rule, local about the point of inoculation, and caused only a slight injury to the plant as a whole. They were usually reddish in color, and extended a half inch or more up and down the stem.

Negative results were obtained with the following species: hyacinth bean (*Dolichos lablab*), white-flowering lupine (*Lupinus polyphyllus*), kudzu vine (*Pueraria hirsuta*), cowpea (*Vigna sinensis*), yard-long bean (*V. sesquipedalis*), soybean (*Glycine max*), broad bean (*Vicia faba*), field pea (*Pisum sativum* var. *arvense*), and sweet clover (*Melilotus alba*). On the green pods of the cowpea a small pustule sometimes formed, as though infection were going to take place, but this vanished shortly, with no further sign of the disease. The bacteria introduced as the inoculum evidently caused a slight disturbance, but, since they were unable to establish themselves and develop, the slight lesion did not persist any longer than the introduced bacteria did. Several attempts were made with this organism to obtain infection of seedlings of the hyacinth bean and the white-flowering lupine, since *Phyt. phaseoli* readily produced diseases in these two species. Under the conditions of the inoculation experiments, however, the organism under observation remained non-pathogenic. The host range of the two pathogenes appears to be different.

ETIOLOGY

Morphology

The pathogene, when grown on beef-extract agar (pH 6.9), is a fairly large rod with rounded ends, occuring singly or in pairs. Chains are seldom found, but during the early hours of growth long filaments are not uncommon. Individual cells stain well with carbol fuchsin and gentian violet; granules are observed at the center of the rod, or in some cases they may be polar. A culture twenty-four hours old on the above medium, grown at 27° C., showed the following measurements: 2.34μ (1.35 to 4μ) by 0.98μ (0.6 to 1.35μ).

The organism does not form spores, and capsules have not been demonstrated. When grown on a medium containing dextrose, a large amount of gum is produced. The organism is motile, but those cultures with which the writer has worked have not been actively so. One polar flagellum has been demonstrated by the use of Loeffler's stain.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated)

Beef-extract agar slants (pH 7.0). In twenty-four hours there is fair yellow growth along the streak, which by the third day becomes abundant, filiform, wet-slimy, and mustard yellow in color; the color is denser in the center and is pale and watery at the edges. The consistency of the culture is frequently watery to thin butyrous. On the second day a slight purplish color is noticeable in the medium. This gradually deepens through a brownish purple to a deep brown. The rate of formation of this pigment appears to vary somewhat with the medium.

Beef-infusion agar slants (pH 7.0). Growth is similar to that on beef-extract agar. The coloration of the agar varies with the lot of media used, from a light to a very deep brown. On one lot which colored very rapidly, the viability of the organism was lost within a week.

Beef-extract agar plates (pH 6.9). Colonies two weeks old are approximately 1 centimeter in diameter, are honey yellow in color, and often have a zone of a watery appearance about the edge. The edges are entire to slightly undulated. In consistency the colonies are watery to weakly butyrous. The yellow pigment of the cells appears somewhat dull, owing to the brown coloration of the medium.

Beef-extract bouillon (pH 7.0). The broth becomes cloudy in twenty-four hours. At the end of four days a light yellow ring develops and a slight flocculation appears in the medium. Small bacterial islands float on the surface and a yellow sediment is formed. Within a week the broth becomes brown near the surface and the color gradually extends downward. At the end of three weeks the medium is a dark brown throughout and the cloudiness of the culture clears to a considerable extent.

Gelatin stab (pH 6.6). (Temperature 21° C.) In twenty-four hours there is a light growth along the stab. Liquefaction begins in three days but is not complete until the end of five weeks. No consistent form of liquefaction occurs. At the end of seven days a browning of the liquefied medium takes place and the pigment progresses with liquefaction. A heavy yellow sediment is formed.

Milk. A yellow ring is noticeable in four days. In six days the milk is yellowish, and a clearing begins at the surface and proceeds rapidly throughout the tube. A heavy casein sediment is formed in the bottom, which is gradually digested. The liquid above is yellow and fairly clear, but later a brown color begins to appear at the surface of the medium and extends downward. After six weeks or more a careful examination will reveal small masses of tyrosine crystals which appear to be partly digested.

Litmus milk. A yellow ring is formed in four days, and a clearing of the milk begins at the surface and rapidly progresses downward. Litmus is reduced, and the liquid becomes rather muddy but later clears and is

purplish in appearance. The brown pigment is then formed. At the end of six weeks the upper half of the medium is a clear purple-brown liquid, but the lower part is muddy in appearance.

Brom cresol purple milk. A yellow ring is formed in four days. On the fifth day a clear purple layer of liquid is produced at the surface, and on the eighth day the entire medium is clear and is a reddish purple by transmitted light. It is difficult to determine whether or not a brown pigment is formed. In six weeks the upper part of the tube is a clear wine-colored liquid but the lower part is muddy.

Uschinsky's solution. On the tenth to the twelfth day a light clouding appears in the medium. There is no further development at the end of six weeks.

Fermi's solution. There is no growth.

Cohn's solution. There is no growth.

Potato cylinders. In twenty-four hours a good watery growth, primrose yellow in color, appears along the streak and soon covers the entire surface. The growth appears slimy, and after a week's time the potato cylinders begin to dissolve. A slight brown color appears and the tuber assumes an olive-ocher color. At the end of five weeks certain tubes are almost black, while others are only a light brown. This is possibly owing to differences in cylinders from different potato tubers.

Cellulose digestion. A good growth appears in the broth, but there was no indication that the filter paper is attacked. A brown coloration appears both in the liquid and on the paper above the broth.

Starch agar plates (pH 6.6). Plates of starch agar streaked five or six times, and tested for starch digestion with a saturated solution of iodine in 50-per-cent alcohol, show a slight digestion of starch at the end of two days and an excellent digestion at the end of six days.

Dextrose broth. The broth in the open arm of a fermentation tube becomes cloudy in twenty-four hours, and later excellent growth is produced. At the end of seven days the brown coloration is distinctly noticeable. No acid nor gas is produced.

Saccharose broth. There is a light cloudy growth in twenty-four hours. A brown color appears within ten weeks. No acid nor gas is produced.

Lactose broth. In twenty-four hours there is a light cloudy growth in the open arm of a fermentation tube. A brown color develops later. No acid nor gas is produced.

Maltose broth. There is a cloudy growth followed by the production of a brown color in the open arm of a fermentation tube. No acid nor gas is produced.

Czapek's agar. An abundant, watery, pale yellow growth is produced, but no brown color is formed in the medium. The production of gum is very noticeable.

Shake agar culture. When a beef-extract agar with 1 per cent of glucose (pH 7.0) is used, there is only a surface growth and no colonies are formed in the depth of the medium.

Indol production. A good cloudy growth is produced in trytophane broth. At the end of one and of four days, tests made for indol by the Ehrlich-Böhme method show no trace of this substance.

Nitrate reduction. A good cloudy growth is produced in nitrate broth but nitrates are not reduced to nitrites. Tests were made at the end of two, four, and eight days with sulfanilic acid and a-naphthalamine in acetic acid. The brown pigment is formed in this medium.

Hydrogen-sulfide production. No growth is obtained on Wilson's medium. On lead-acetate agar a browning occurred along the stab, but this brown coloration appeared to be the usual coloration produced by this organism on most media. It is doubtful whether hydrogen sulfide is produced.

Chromogenesis. The brown water-soluble pigment which is formed in most media, owing to the action of the pathogene, is so striking in appearance that it demands a certain amount of attention. It is a phenomenon not commonly met with among the members of the bacterial plant pathogenes, although it occurs not infrequently with species of the genus Actinomyces. A brown pigment has been reported in literature as being produced by *Phytomonas campestre* and *Phyt. hyacinthi*, but most descriptions of these organisms do not refer to it; and a sufficient description has never been given to permit of determining whether or not it is similar to the pigment formed in media by the action of the bean pathogene. It is possible, too, that only certain strains of the two bacteria referred to are capable of causing the formation of the brown coloration of the media. The information is rather scanty.

The brown coloration formed in cultures of certain species of Actinomyces appears to be the same or a closely related product. With this group of organisms the phenomenon is considered to be the result of an enzymatic action on the amino acid, tyrosine, contained in the media, and the production of melanin. It is probable that two enzymes produced by the organism are implicated in the process. Waksman (1926) has reviewed the literature on tyrosinase and has suggested the possible chemical reactions which result in the brown substance formed. It will be noted in the above cultural descriptions of the organism that the brown color does not appear in synthetic media where tyrosine would be lacking.

Acting on this suggestion, Conn's (1921) synthetic medium for growing species of Actinomyces was prepared. In one lot of this medium, tyrosine replaced the sodium asparaginate. The organism grew fairly well on the two simple media, and on the second day a purple-brown color appeared in the tyrosine agar and deepened to a dark brown. No color appeared in the sodium-asparaginate agar. For further confirmation, *Phyt. phaseoli* was grown at the same time on the two media. After three weeks of incubation a light brown color did appear on the tyrosine agar, but this was considered to be due to a natural breaking-down of the amino acid under these conditions. One-tenth of 1 per cent of tyrosine had been used in this medium, which, no doubt, is an excessive amount. The difference

in the two cases, however, was distinct. The organism under consideration produced a deep color, which began to be formed on the second day of incubation. Color did not appear in cultures of *Phyt. phaseoli* until after three weeks.

A further experiment, similar to the one just described, was conducted, using, in place of Conn's medium, the synthetic medium suggested by Ayers, Rupp, and Johnson and modified in the *Manual of Methods* of the Society of American Bacteriologists (1923–29). Dextrose was used as the sugar. There was no substitution in the nitrogen source, but to one lot of media 0.1 per cent of tyrosine was added. In no case, however, did a brown color develop on the agar. It is possible that there was an inhibiting factor present. Just what this inhibition is, was not determined, although it has been noticed that in beef-extract agar to which dextrose has been added, the color is somewhat slow in developing, and the amount is never great. This sugar may be accountable for the failure of the brown color to appear in the above-named synthetic medium.

That this brown water-soluble pigment in the various media is due to the action of an enzyme or enzymes upon tyrosine seems evident, however, from the following facts: First, the color appears in media containing peptone, potato, or some other substance, where the tyrosine would probably be present, and in milk where it could be formed. Secondly, no brown color develops in a synthetic medium in the absence of tyrosine. Thirdly, such a pigment develops in Conn's medium when tyrosine is substituted for sodium asparaginate. Fourthly, in milk cultures that have begun to show a brown pigment, masses of tyrosine crystals may be found which are partly digested. It is probable that the pathogene, acting upon casein, produced tyrosine, which in turn is acted upon by a further enzyme or enzymes.

Longevity. No carefully planned experiments have been conducted on this phase of the problem, but certain observations are of interest. On beef-extract agar and broth, many of the strains die within a month. Cultures usually are transferred every two to three weeks to insure their remaining viable. The browning and clearing of broth cultures often is accomplished by death. On beef-infusion agar, where the brown pigment appears early and becomes intense, many cultures die within a week. There appears to be a correlation between the production of the brown color and death of the culture. Old potato cultures, however, have revealed viable organisms.

Nomenclature

Although the production of this brown pigment in various media is a very striking character of the pathogene, it is the only cultural characteristic so far noted which separates the organism from *Phyt. phaseoli.* The formation of filaments is a slight, but distinct, morphological character,

and in the symptomatology and in the host range there are several differences, the last two distinctions of which have already been discussed. It is felt that these differences are probably varietal and not specific. Therefore the name *Phytomonas phaseoli* var. *fuscans* n. var. is given to the pathogene.

Brief description

Phytomonas phaseoli var. *fuscans* is a non-spore-forming rod with rounded ends, occurring singly or in pairs. The cells measure 1.35 to 4 μ by 0.6 to 1.35 μ. Filaments are not uncommon. The organism is motile by means of a single polar flagellum. It stains well with carbol fuchsin and gentian violet, showing granules. It is Gram-negative, not acid-fast, and an aerobe.

The colonies on nutrient agar are yellow, fairly rapid-growing, in many cases with a watery-appearing zone about the edge. The edges are entire to slightly undulating. The consistency of the culture is watery to butyrous; most media are colored brown; the broth is cloudy in twenty-four hours, a yellow ring is formed, and the medium becomes brown at the surface, the brown color extending gradually throughout; gelatin is liquefied and colored brown; milk is alkaline and cleared, and later a brown pigment is formed; there is no acid nor gas in dextrose, saccharose, lactose, or maltose broth; starch is hydrolyzed; nitrates are not reduced to nitrites; indol is not produced; hydrogen sulfide is not formed on lead-acetate agar; there is no growth on Wilson's agar, in Cohn's solution, or in Fermi's solution; growth in Uschinsky's solution is slight; the thermal death-point is approximately 49° C. The viability of the organism in culture is not great.

The organism is pathogenic on the common bean (*Phaseolus vulgaris*), the sieva bean (*P. lunatus*), and the scarlet runner bean (*P. coccineus*).

THE BACTERIAL WILT OF THE BEAN CAUSED BY PHYTOMONAS FLACCUMFACIENS

The bacterial wilt of the bean was reported first by Miss Hedges (1922), who later (1926) published a fuller account of the disease. Leonard (1923, 1924) also has reported on various phases of the wilt. The disease is of widespread distribution in the United States, and in certain sections causes considerable loss. In New York State, although it has been known for some time, it has never become of as great economic importance as have some others of the bacterial diseases. It does occur, however, generally throughout the sections growing dry shell beans, and may be found on such varieties as Red Kidney, Pea, and White Marrow.

LIFE HISTORY

In many respects the life history of *Phytomonas flaccumfaciens* is very similar to that of *Phyt. phaseoli*. There are, however, certain differences, since *Phyt. flaccumfaciens* is more limited in its activities on the host plant.

The overwintering of the bacteria takes place in the seed, and in this

manner it is introduced into the field in the following year. There may be other methods by which the pathogene may survive the winter, but they are probably not important in its life history. The production of diseased plants from slightly infected seeds can be demonstrated easily, and infected seeds are sometimes frequent in seed beans. On the other hand, no work has been done to determine whether or not the bacteria can overwinter in the soil or in refuse. In a dry condition in the seed, and even in the leaves, of its host plants, the organism is extremely resistant. It has been isolated from dried leaves kept in the herbarium for five years, and from seed stored in the laboratory for a similar period of time. This duration of time is possibly not the limit of its viability period, but further tests have not been made. Infected seed treated at a temperature of 100° C. in a hot-air oven for an hour still yield pathogenic cultures.

The activities of *Phyt. flaccumfaciens* in relation to its host are as follows: When an infected seed germinates in the soil, the bacteria become active and pass from the cotyledons into the stem. Here they may be found in great numbers in the tissue of the xylem vessels, for which they show a decided preference. This organism, as well as *Phyt. phaseoli*, passes through an entire plant in the vascular system. Other tissue besides the xylem seldom is infected, and the organism appears to lack the ability to invade such tissue. In spreading from one bean plant to another, however, there is some difficulty. The bacteria can produce an infection only through mechanical injury, as Miss Hedges (1926) has shown. Consequently, rain splashing the bacteria from plant to plant would be of no importance unless some injury had already occurred in the plant that was spattered. In many bean fields, insects of both the leaf-chewing and the sucking types are found commonly, and probably are an important factor in the spread of the pathogene. Experimental evidence on this point, however, is lacking.

In completing their life history, the bacteria must again infect the seed. They do this, in a large majority of cases, by passing into the seed through the vascular system. The entrance of *Phyt. flaccumfaciens* through the side of the pod and the ultimate invasion of the seed, as is a common occurrence with *Phyt. phaseoli*, rarely happens. *Phyt. flaccumfaciens*, when introduced into the fleshy tissue on the side of the pod, does not cause infection, or, at the most, produces only a very slight lesion (Plate III, A). When the injury extends into the pod cavity, however, the bacteria may multiply there, although they cause scarcely any discoloration of the pod tissue. Under such conditions, bacteria have been observed to grow on the surface of a seed while the seed matured normally. If the seeds are injured during inoculation, they become infected and rot. Seeds infested with the bacteria on their surface may give rise to diseased seedlings.

When *Phyt. flaccumfaciens* enters the pod through the vascular system of the pedicel, a suture lesion usually is produced (Plate IV). Since otherwise this pod tissue does not become infected, the suture lesion appears to be the result of an overflowing of the bacteria from the xylem vessels.

SYMPTOMS

General

Since *Phytomonas flaccumfaciens* is primarily a vascular parasite and invasion has a decided tendency to become systemic in nature, the general symptoms of a diseased plant are the most pronounced. A stunting of an infected plant takes place and, as a rule, quickly develops into a wilt. It is this wilting of an infected plant, which is the most outstanding symptom of the disease, that led Miss Hedges (1926) in first describing the disease to give to it the common name *wilt*. At any stage in the development of the plant the wilt may take place, from a seedling wilt to the wilt of the entire plant bearing mature pods. Wilting of a part of the plant, as a branch, also is not uncommon. Since the stalk and the branches of a maturing bean plant are more or less woody in nature, the true flagging is limited primarily to the leaves and the young growing shoots. Even the leaf petioles seldom droop, but remain erect. Wilted leaves become dry and brown, and, after several rains, very ragged. Sometimes the leaves on an infected plant will not persist but will drop off.

On the leaf

As already stated, the wilt is the primary symptom of this disease, and this is especially true with reference to the leaves. The leaflets may flag, beginning at the tip or along the side. Such leaves fail to recover, dry up, and turn brown, still persisting on the plant. A heavy wind or rain may destroy these leaves completely or whip them to a very ragged condition. In some cases, but less frequently, only a part of the leaf may wilt and die, thus giving rise to a rather characterless lesion as shown in figure 5.

In some plants, the vascular systems of which are infected, the leaflets may turn somewhat yellow, dry up, and fall off. In such cases the main petiole remains standing erect.

On the stem

There is probably only one type of lesion found on the stem of bean plants infected with *Phyt. flaccumfaciens*. This is a stem girdle, or a disease of the nodes. These lesions appear to be of endogenous origin, the bacteria working their way out from an infected xylem along a leaf trace. Such infected nodes turn red, and are greatly weakened so that the plant is broken off easily during a windstorm. The stem girdle is not peculiar to this disease, but may be caused by *Phyt. phaseoli* and possibly by *Phyt. medicaginis* var. *phaseolicola*.

On the pods

Lesions produced on the pods by *Phyt. flaccumfaciens* occur along the suture. The bacteria spread from the fibrovascular bundles, causing a

FIGURE 5. PRIMARY LEAF OF SIEVA BEAN (PHASEOLUS LUNATUS) SHOWING LESIONS RESULTING FROM INOCULATING THE STEM OF THE PLANT WITH PHYTOMONAS FLACCUMFACIENS

dark green, water-soaked spot sometimes extending the entire length of the pod (Plate IV). Lesions produced in the greenhouse retain this green, water-soaked appearance even after the remaining part of the pod has lost its chlorophyll, dried, and turned white. In such a state they appear dark green and rather gummy. It is not known whether or not the lesions under natural conditions in the field ever dry out and turn reddish brown, as do the water-soaked spots of *Phyt. phaseoli*. This point is yet to be determined.

On the seeds

The symptoms produced by *Phyt. flaccumfaciens* on the seeds of beans are practically the same as certain of those produced by *Phyt. phaseoli*. The bacteria entering the seed through the vascular system may produce a yellow spot on the hilum. If infection is more severe, the organisms spread out underneath the seed coat and discolor the seed. If the seed is white, infection with this pathogene may turn it a bright yellow. Severely infected seed may be very wrinkled and appear as if varnished.

INOCULATION EXPERIMENTS

All of the symptoms described above have been produced on the Red Kidney bean in the greenhouse by artificial inoculation. Infection always is obtained at a temperature of 80° F., provided the plant is injured and the injury extends into the fibrovascular bundles. Red Kidney is very susceptible and is one of the best varieties to use for inoculation purposes.

Cross-inoculation experiments were conducted on thirteen species of legumes, eight of which proved to be highly susceptible. *Phytomonas flaccumfaciens* thus appears to have a particularly wide host range, and there are, no doubt, other susceptible species which are still unknown. The inoculations were made by injuring a seedling or a young, vigorously growing plant on the stem, and introducing the pathogene at the same time. The symptoms resulting on the susceptible species were more or less similar to those appearing on the common bean (*Phaseolus vulgaris*); that is, a wilt occurs, either of the entire plant or of a part of it. *Phyt. flaccumfaciens* remained in all cases a vascular parasite. The susceptible species are as follows: sieva bean (*P. lunatus*), scarlet runner bean (*P. coccineus*), adzuki bean (*P. angularis*), cowpea (*Vigna sinensis*), yard-long bean (*V. sesquipedalis*), soybean (*Glycine max*), and hyacinth bean (*Dolichos lablab*).

Negative results were obtained with the following species: white-flowering lupine (*Lupinus polyphyllus*), kudzu vine (*Pueraria hirsuta*), broad bean (*Vicia faba*), field pea (*Pisum sativum* var. *arvense*), and sweet clover (*Melilotus alba*). Since infection always was obtained when susceptible species were inoculated, it is felt that this list is correct under the conditions used. When infection was not obtained at the first inoculation, as on these five species, the experiments were repeated two or three times. These inoculations involved from twenty-five to fifty individuals in all.

ETIOLOGY

Miss Hedges (1926) has already published a very excellent description of this organism, and the writer's description, presented here, is included principally to give opportunity for a comparative study with the other bacteria which cause blights on beans. Ten strains isolated from various places in New York State were used in preparing the following description.

It was found that certain strains seemed to vary from the original, and these variations are pointed out. *Phytomonas flaccumfaciens* appears not to be as stable a species as *Phyt. phaseoli.*

Morphology

Phyt. flaccumfaciens is a short rod with rounded ends, non-spore-forming, occurring singly or in pairs. The longest rods often show a slight curve, and a few of the cells appear to be larger at one end than at the other. Grown on beef-extract agar (pH 6.9) at a temperature of 27° C., the cells of a culture twenty-four hours old measured: 1.75μ (0.9 to 2.7μ) by 0.82μ (0.6 to 1.2μ). Granules have been observed in the cells.

The organism is motile by means of a single polar flagellum. It is not acid-fast, but is Gram-positive. One strain proved to be Gram-variable, and even grew well in gentian-violet agar. Capsules have not been observed.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated)

Beef-extract agar slants (pH 7.0). Growth appears in twenty-four hours. The growth is never more than moderate, is filiform, smooth, translucent, and straw yellow in color. The color appears to vary with the lot of media. In some cases there is scarcely any pigment production until after three or four days, and then it may be very weak. In other cases the pigment may be deep and similar to that of *Phyt. phaseoli.* The consistency is often viscid.

Beef-infusion agar slants (pH 7.0). Growth is similar to that on beef-extract agar, although sometimes it is more copious. The color is more intense on this agar, and becomes in several days a light cadmium.

Beef-extract agar plates (pH 7.0). Seven-days-old colonies are circular, approximately 3 to 5 millimeters in diameter, and mustard yellow in color. The consistency is slightly stringy.

Beef-extract bouillon (pH 7.0). The medium becomes slightly cloudy within twenty-four hours. Growth advances somewhat, but there is never more than a moderate cloudy appearance. A light sediment is formed.

Gelatin stab (pH 6.6). (Temperature 21° C.) Growth appears along the stab within twenty-four hours. Liquefaction varies with the strain, and may begin within three days to three weeks. In some cases liquefaction has been complete in five weeks. In certain lots of gelatin, several strains have shown no liquefaction at all.

Litmus milk. Litmus milk begins to turn pink on the fourth day. A distinct yellow ring is formed, and after a week's time reduction of the litmus commences. After four weeks a soft curd is formed, which becomes solid, and in some cases a slight peptonization takes place. Many variations of this procedure may occur: in litmus reduction, from

complete to slight; in curd formation, from none to a solid; and in the amount of peptonization.

Brom cresol purple milk. A slight yellow-green color appears in about six days, and later a yellow ring is formed. At the end of four weeks, a very soft curd may appear and slight peptonization begins. At the end of eight weeks, the cultures are a greenish brown in color.

Uschinsky's solution. No growth was observed.

Fermi's solution. No growth was observed.

Colin's solution. No growth was observed.

Potato cylinders. As a rule, growth appears in twenty-four hours along the streak. It is a light to fair growth, and is pale yellow at first, deepening in seven days to a mustard yellow. The medium may or may not be darkened. Certain strains will scarcely grow on potato. One strain produced a vigorous growth covering the entire cylinder in five days, and was a deep aniline yellow in color. The medium remained unchanged.

Cellulose digestion. A very light, cloudy growth appears in the liquid, but there is no sign of cellulose digestion.

Starch agar plates (pH 6.6). Macroscopically there is no indication of starch digestion. Miss Hedges, however, reports that when the cultures are examined microscopically, a slight digestion may be observed. In making determinations, plates were streaked five or six times, and tests for digestion of starch were made at the end of two, six, and ten days. The tests were made by flooding the plates with a saturated solution of iodine in 50-per-cent alcohol.

Dextrose broth. A cloudy growth appears in the open arm of a fermentation tube within twenty-four hours. A color reaction due to acidity appears in two to three days. The intensity of the reaction varies with the strain, and in all cases the color appears to deepen with age. The following table shows the variation in hydrogen-ion concentration in dextrose broth due to ten different strains. The strains are listed in the order in which they were isolated.

Strain	Original pH	pH after seven days	pH after fourteen days
6	7.0	6.50	5.90
17	7.0	6.00	5.00
24	7.0	6.45	5.10
30	7.0	6.35	5.80
31	7.0	6.55	5.20
32	7.0	6.45	5.85
33	7.0	6.50	6.45
33–5	7.0	6.35	5.45
B	7.0	6.05	5.00
O	7.0	6.15	6.15

Saccharose broth. A cloudy growth appears in the open arm of a fermentation tube within twenty-four hours. The medium soon becomes acid, and the reaction becomes greater with age. No other change occurs.

Lactose broth. A light, cloudy growth appears in the open arm of a fermentation tube within twenty-four hours. On the second day the medium shows an acid reaction. From a light pink in color, it gradually deepens to a distinct red.

Maltose broth. A light, cloudy growth appears in the open arm of a fermentation tube within twenty-four hours. A slight acidity appears on the fourth day and gradually increases in intensity. No other changes occur.

Indol production. The organism does not produce indol when grown in trytophane broth, as shown by the Ehrlich-Böhme test used at the end of one and of four days.

Nitrate reduction. The organism grows well in nitrate broth, and tests made at the end of two, four, and eight days with sulfanilic acid and a-naphthalamine in acetic acid show no reduction of nitrates to nitrites.

Hydrogen-sulfide production. A very light growth is obtained on Wilson's medium, and in certain cases there has been no growth. Where growth does occur there is no positive reaction for hydrogen-sulfide production. Growth occurs in lead-acetate agar stabs with all strains tested. Certain strains show a slight browning along the stab, while others remain colorless. Hydrogen sulfide is not produced, or is produced only weakly by certain strains.

Chromogenesis. The organism is pale yellow in color, but the intensity of the pigment may vary with the strain and the medium used. The color is more intense on beef-infusion agar, and is almost absent on dextrose agar.

Oxygen relations. Although the organism ferments all the sugars tested, there is no sign of growth in closed arms of fermentation tubes. In shake cultures of beef-extract agar with 1 per cent of dextrose, colonies appear 5 millimeters below the surface. On the whole, the pathogene appears to be a fairly strict aerobe.

Longevity and virulence. This pathogene is very resistant to adverse conditions, and remains viable in culture for a long period of time. It has been isolated from dried leaves which have been in the herbarium for five years. Once in culture, it does not lose its pathogenicity or its virulence to any appreciable extent. Old cultures which are very dry will often yield viable cells. The organism may be recovered from bean seeds infected with it and treated with dry heat at 100° C. for an hour. It does not produce spores in culture, and probably does not in nature. Partly protected by the bean seed, and being in a dry condition, it is highly resistant to dry heat.

Thermal death-point. Tests have not been made to determine the thermal death-point, but Miss Hedges reports that it is approximately 60° C.

Brief description

Phytomonas flaccumfaciens is a short, non-spore-forming rod with rounded ends, occurring singly or in pairs. The average size of the cells is 1.75 by 0.82μ. The organism is motile by means of a single polar flagellum. It is Gram-positive, not acid-fast, and apparently a fairly strict aerobe. Capsules have not been observed.

On agar slants the growth is moderate, filiform, and pale yellow in color. The consistency of the culture is often viscid; broth cultures are cloudy in twenty-four hours; gelatin liquefaction, as a rule, is light; a soft curd is formed in milk; litmus is reduced; acid is produced, but no gas, in dextrose, saccharose, lactose, and maltose broth; starch is not hydrolyzed or but slightly; nitrates are not reduced to nitrites; indol is not formed; hydrogen sulfide is not produced; there is no growth in Uschinsky's, Fermi's, or Cohn's solution; pathogenicity or virulence is not lost in culture.

The organism is pathogenic on the common bean (*Phaseolus vulgaris*) and on many related species.

HALO BLIGHT CAUSED BY PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA[1]

The bacterial disease of greatest economic importance in New York is the one caused by the organism *Phytomonas medicaginis* var. *phaseolicola*. This disease was first noticed in 1924, and, because of the severity of its attack, suspicion immediately arose that it was distinct from the other known bacterial diseases of beans. The rapidity with which the disease spreads through a field, and the severity of the symptoms on the individual plants, are still undiminished.

In that section of New York where the Red Kidney bean is grown extensively, this disease has been in the past few years a limiting factor to production. It may be differentiated on this variety from the other bacterial diseases almost alone by the severity of its attack, regardless of the types of symptoms. On the other hand, the Pea bean, the Marrow, and the Yellow Eye, of the dry-shell varieties, and the Refugee of the green-pod types, are not so susceptible as the Red Kidney, although they should never be considered resistant.

The distribution of the disease, and the extent of its economic importance outside New York, have never been carefully investigated. Apparently it is of widespread occurrence. Miss Hedges (1928) has reported it from Georgia and Montana, and she states that in those sections it causes considerable losses to the crop. In 1927 the writer isolated the causal organism, *Phyt. medicaginis* var. *phaseolicola*, from specimens collected in Switzerland, showing that the disease it is not limited entirely to America. It probably is of widespread distribution, but its general similarity to the

[1] In a recent publication (Phytopath. 20:140. 1930), Miss Hedges states that *Phytomonas pueraria* is a synonym of *Phyt. medicaginis* var. *phaseolicola*.

diseases produced by *Phyt. phaseoli* and *Phyt. flaccumfaciens* has caused it to be overlooked as a separate entity.

The symptoms of the disease caused by *Phyt. medicaginis* var. *phaseolicola* have been described, but perhaps not fully. Careful observation of this disease in the field has been maintained during the past five years, and many variations of the symptoms have been noted. It has been observed that certain types of lesions, especially on the leaves of the host plant, occur in one season in profusion and in the following season are inconspicuous. These variations in symptoms appear to be due to ecological factors, and no indications have been observed that various strains of the pathogene are involved. Consequently, after these observations over a considerable period of time, a better picture may be given of the disease as it appears under natural conditions in the field than was given in the first report.

In the following descriptions of the symptoms of the disease, it must be remembered that observations have been made primarily in the field on the Red Kidney variety of beans. The symptoms do not differ, however, except in intensity, on such common varieties as Pea, Marrow, Yellow Eye, White Kidney, and Refugee. In the majority of cases the symptoms observed in the field under natural conditions can be reproduced in the greenhouse.

LIFE HISTORY

The life history of *Phytomonas medicaginis* var. *phaseolicola* is very similar in detail to that of *Phyt. phaseoli*, and for this reason only the salient points are given here.

The organism overwinters on and in the bean seed, and becomes active in the spring when such seeds are planted and have germinated. Sufficiently high temperature and enough moisture are necessary for the abundant development of the bacteria in the cotyledons. Here they multiply rapidly in the host tissue, and may serve as inoculum to be splashed by rain to the leaves and other parts of the infected individual or to near-by plants. The organism evidently gains entrance to the plant through the stomates, since mechanical injury is not necessary for infection to take place. A second mode of invasion is for the bacteria to enter the plant from the cotyledons by way of the vascular system. Under such conditions an infection of the vascular system takes place, the xylem being the tissue most generally invaded although the bacteria may break forth into the adjacent cells and even to the surface of the plant. Since *Phyt. medicaginis* var. *phaseolicola* exhibits a greater virulence than does *Phyt. phaseoli*, the disease from this last-named type of invasion is more likely to become acute, rather than remain dormant for some time as it does with *Phyt. phaseoli*. A further difference between the two organisms is that *Phyt. medicaginis* var. *phaseolicola* appears to produce a toxin which precedes the organism in its harmful effect on the plant. This is not so noticeable with *Phyt.*

phaseoli. The two organisms are alike, however, in the tissue which they attack. Each shows a preference for the vascular tissue, and uses the xylem vessels as a means of spreading throughout the plant. They can, on the other hand, attack the other tissues of the plant with great vigor, although they do not spread so rapidly in these.

One point in the life history of the organism of which little is known is its ability to overwinter in the soil. In the greenhouse it is known to live for several months in garden loam, but no carefully conducted experiments have been carried on in the open. From observations in the fields of New York, it is evident that clean seed, such as is brought in from California, produces, as a rule, healthy crops of beans. Since the crop rotation in the bean section, however, is seldom less than three years, it would be necessary for the pathogene to remain viable in the soil for that length of time in order for this phase of its life history to be of any economic importance. There is no indication that the organism possesses this ability.

SYMPTOMS

Stunting and wilt

It has been pointed out, in the discussion of the life history of *Phytomonas medicaginis* var. *phaseolicola*, that this pathogene, while not obligatively a vascular parasite like *Phyt. flaccumfaciens*, nevertheless does show a decided tendency to invade the xylem vessels. The result of the infection of the xylem tissue is, in most cases, a symptom complex, and should be regarded as such rather than as isolated individual symptoms. The latter is the case only when the infection is local. The symptoms arising from the infection of the xylem are definite, and in certain respects are peculiar to this disease. A seedling wilt, however, may result which is not peculiar to this organism and which cannot be distinguished from such a wilt induced by other bacterial pathogenes. In inoculation experiments this wilt takes place in about four days, but it is not of frequent occurrence in the field. Possibly the reason for this is that the seeds which are planted have been graded in a seed house, where the severely infected seed are removed and only the lightly infected ones remain; consequently, the bacteria are not present in sufficient quantity to enable them to reach the seedling from the cotyledon and cause a wilt at this early stage of development. Young plants with infected xylems, however, will soon show certain definite symptoms of the disease. They are stunted in growth, and as the pathogene progresses upward in the stem the lower leaves are invaded and soon wilt. The leaf petiole in this case remains upright, while the leaflets droop downward, become dry and brown, and persist until they are destroyed by wind or rains. As the disease progresses upward, the remaining leaves, the young shoots, and the immature pods succumb. The upper leaves and shoots of an infected plant are stunted in their development, and become chlorotic before they finally are killed. From this it appears that the pathogene in the xylem vessels produces a toxin

which moves ahead of the bacteria and causes an injury in the still uninfected part of the plant. At least the pathogene cannot be isolated from the chlorotic tissue beyond the microscopically visible advancing line of bacteria.

On the leaf

There are two types of symptoms produced on the leaves of beans by *Phyt. medicaginis* var. *phaseolicola*, namely, those due to a systemic infection and those due to a local infection.

The most striking symptom arises from a local infection, and is the spot to which Miss Hedges has applied the term *halo blight*. The spot, which varies in size, may become necrotic, dry, and brown, and appear indistinguishable at the center from other necrotic leaf spots due to the

FIGURE 6. THE "HALO" LEAF SPOT OF THE BEAN CAUSED BY PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

various bacteria described in this memoir. The peculiarity, however, which sets it apart from the leaf spots of the other diseases, is a chlorotic area, or zone, about the dead part. This zone is very pale green, and varies in width from a half inch to an inch. The zone naturally changes with the size of the necrotic area, but a very small spot may show a surprisingly large zone (figure 6). A leaf spot of this type is a fairly good diagnostic symptom, but probably is not infallible. The halo spot is found, as a rule, during cool weather, and later in the season, during the hot weather, it gives place to another type of lesion. The lesions of this latter type are numerous on the leaf, and are small and angular (figure 7).

FIGURE 7. A TYPE OF LEAF SPOT OFTEN PRODUCED BY PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

A chlorotic zone is not so evident, although the lack of a halo is due partly to the fact that the whole leaf is somewhat chlorotic. Nevertheless it is very noticeable that a halo spot is never produced in the hot days of July and August. The angular spots just described probably arise from stomatal infection, and in most cases are an infection of the islets between the veins.

Other necrotic leaf spots due to this organism may arise, similar in

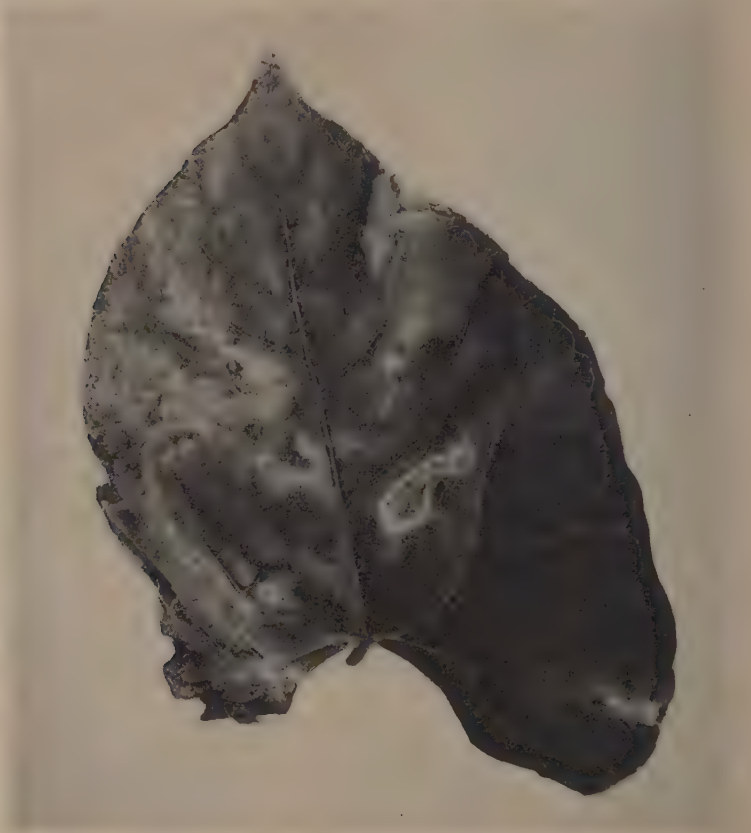

FIGURE 8. PRIMARY LEAF OF A BEAN PLANT WHICH WAS INOCULATED AT THE COTYLEDON NODE WITH PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

appearance to the ones commonly found in plants infected with *Phyt. phaseoli*. Such spots may vary in size and become fairly large (figure 8). They are not angular, and have no definite, wide, pale green zone although they may be surrounded by a narrow yellowish border. These spots often arise from infections of endogenous origin in systemically infected plants.

FIGURE 9. LEAF OF A WHITE MARROW PLANT SHOWING MOTTLING DUE TO PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA IN THE VASCULAR SYSTEM OF THE STEM AND THE PETIOLES

When the infection is local and arises from external infection, the xylem may or may not be involved.

With plants in which the bacteria have multiplied rapidly in the xylem vessels and have become very numerous, certain symptoms appear on the leaves before the pathogene itself has extended to them. There is first a definite mosaic appearance (figure 9), with lighter and darker areas distributed over the leaf, the darker areas being nearer the larger veins. This symptom has been mistaken for the true mosaic, but necrotic lesions appearing on the plant soon make evident the difference. The mosaic-like symptom is not difficult to produce in the greenhouse in artificially inoculated plants.

A more severe manifestation of this type of chlorotic symptom has been noticed frequently in the field on Red Kidney beans. In this case the young leaves of an infected plant are stunted in size, very pale green in color, and extremely crinkled, and the veins are very prominent. Such plants usually succumb very quickly to the disease.

On the stem

Longitudinal streaks due to this pathogene may appear on the stalk, the stem, and the leaf petioles of the bean. On seedlings, the tissue of which is very tender and succulent, such lesions at first are water-soaked and dark green. Later they may dry down and become somewhat reddish, though the color is not always present. On older plants the water-soaked condition is not so evident. Stem lesions, especially those on the seedlings, are local and usually of exogenous origin. When the stem lesions are of endogenous origin a somewhat different picture is produced. The bacteria breaking through the xylem vessels cause longer lesions, often with longitudinal cracks. One very characteristic condition may occur on such lesions which is not found in any of the other bacterial diseases of the bean. The pathogene may ooze out in relatively large droplets on the surface of the stem (figure 10). These droplets are colorless to milky, and in appearance are not unlike those due to *Erwinia amylovora* in fire blight of apples and pears. As a rule they have been observed in the field in the early part of the growing season and during cool, wet weather. They have also been produced in the greenhouse on Red Kidney plants.

On the pods

The individual spots produced by the disease on bean pods are more or less circular in outline, but they may anastomose and give rise to rather irregular lesions (figure 11). These lesions are at first green and water-soaked, but later they may dry out and become sunken and reddish brown in color. In this respect they are similar to spots produced by *Phyt. phaseoli*. In some cases the water-soaked appearance may still be present even after the pods have ripened and dried. Over these pod lesions one

FIGURE 10. OPAQUE DROPS CONTAINING BACTERIA ON THE SURFACE OF BEAN STEMS SYSTEMICALLY INFECTED WITH PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

may sometimes find a light, silvery crustation due to the bacteria oozing to the surface and drying down. This is a very delicate crust and may be washed away quickly during a rain.

When the bacteria pass through the vascular system of the pedicel and enter the pod, a rather irregular lesion along the suture may be produced (Plate IV, A). Such a suture lesion differs in appearance in no respect from those of the spots as described above, except in position and outline. These lesions are indistinguishable from those produced by *Phyt. phaseoli* and *Phyt. flaccumfaciens.*

On the seeds

This disease may involve the entire seed or only a small part of it. On white seeds the distinguishing characteristic of the spot is the maize yellow to cream color of the affected area. Severely infected seeds are small and wrinkled, and may be completely colored by the pathogene. On colored seeds the spots due to *Phyt. medicaginis* var. *phaseolicola* are

FIGURE 11. PODS OF RED KIDNEY BEAN SHOWING LESIONS CAUSED BY PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA

This photograph would serve equally well to illustrate lesions caused by *Phyt. phaseoli* or *Phyt. phaseoli* var. *fuscans*

impossible to differentiate from those caused by the other bacterial or fungous diseases that affect the bean seed.

Phyt. medicaginis var. *phaseolicola* may infect the seed by working its way through the pod tissue from local infections on the outside, or it may enter the seed through the vascular tissue. In the latter case the bacteria spread out underneath the seed coat. Very light infections of either type are of the greatest importance, since they pass unobserved by the pickers in the seed houses and the beans may be used for seed. A few infected plants in a field are all that is needed for the disease to become epiphytotic when the conditions are favorable.

INOCULATION EXPERIMENTS

Many cross inoculations have been conducted with *Phytomonas medicaginis* var. *phaseolicola* on about a dozen legumes and a few non-leguminous plants. In these experiments the organism has shown itself to be rather restricted in its pathogenicity. Besides the common bean (*Phaseolus vulgaris*), only two members of the genus Phaseolus — the scarlet runner bean (*P. coccineus*) and the sieva bean (*P. lunatus*)—proved to be susceptible. All species outside of this genus used in the cross-inoculation experiments were found to be immune. This is a host range more limited than that of *Phyt. flaccumfaciens* or even that of *Phyt. phaseoli.*

Negative results were obtained with the following species of legumes: adzuki bean (*P. angularis*), mung bean (*P. aureus* Roxb.), cowpea (*Vigna sinensis*), yard-long bean (*V. sesquipedalis*), soybean (*Glycine max*), broad bean (*Vicia faba*), hyacinth bean (*Dolichos lablab*), white-flowering lupine (*Lupinus polyphyllus*), kudzu vine (*Pueraria hirsuta*), field pea (*Pisum sativum* var. *arvense*), red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa*), and sweet clover (*Melilotus alba*). Attempts were made, but without success, to infect the following non-leguminous plants: potato (*Solanum tuberosum* L.), cotton (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill.), and tobacco (*Nicotiana tabacum* L.).

ETIOLOGY

Morphology

Phytomonas medicaginis var. *phaseolicola* is a fairly large rod with rounded ends, occurring singly or in pairs. Single cells are sometimes found which are slightly curved, and filaments are not uncommon on certain media. The cells stain well with carbol fuchsin, gentian violet, and Loeffler's methylene blue. Granules always may be demonstrated, and in some cases they are bipolar. On beef-extract agar (pH 6.9), and grown at a temperature of 27° C., single cells of a culture twenty-four hours old measured as follows: 1.9μ (1.35 to 3.6μ) by 1.0μ (0.9 to 1.2μ).

The pathogene is actively motile and a single polar flagellum has been

demonstrated. The organism does not form spores, is Gram-negative, is not acid-fast, and is a facultative anaerobe.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated.)

Beef-extract agar slants (pH 7.0). Growth is at first scanty to moderate, filiform, translucent, white or slightly creamy, glistening, smooth except at the edges, which often are wrinkled. The consistency is butyrous, but in old cultures it may be brittle. There is no change in the medium.

Beef-infusion agar slants (pH 7.0). The growth is similar to that on beef-extract agar. A green fluorescent pigment has never been observed on this medium.

Beef-extract agar plates (pH 6.9). Three-days-old colonies are approximately 2 millimeters in diameter, white but bluish by transmitted light, smooth with edges entire. Later, the growth often shows a zonation with edges slightly undulating (Plate VI, E).

Beef-extract bouillon (pH 7.0). The broth becomes cloudy within twenty-four hours. A delicate pellicle appears, which is readily broken up and thrown to the bottom. A white granular sediment is formed.

Gelatin stab (pH 6.6). (Temperature 21°C). In the original description of this organism, it was stated that arborescent growth occurs on this medium and that no liquefaction takes place. Further work with the pathogene on other gelatins, however, showed that this is not altogether correct. Tests with one of the original strains and with several recently isolated strains proved the following to be nearer the truth:

Growth appears along the stab in twenty-four hours; this sometimes becomes somewhat arborescent. At the end of two weeks a slight liquefaction begins, which proceeds very slowly. At the end of six weeks the liquefied gelatin is often like a heavy oil. (The mistake in the original description was probably due to the gelatin used.)

Milk. No separation occurs. At the end of six weeks the milk appears somewhat yellowish brown in color.

Litmus milk. In five days the litmus begins to turn blue, and it remains so throughout the experiment. At the end of six weeks the medium is a rather muddy blue.

Brom cresol purple milk. The milk begins to turn blue in five days, and finally becomes a brilliant bluish purple. There is no further noticeable change.

Uschinsky's solution. There is a slight variation with the different strains. A light clouding occurs in forty-eight to seventy-two hours, sometimes with a delicate surface film. A greenish color appears in three to eight days. Many shades of this color may be noticed, but at the end of six weeks all cultures are a light grape-green. The growth is very cloudy to turbid, with a heavy white pellicle and a heavy white sediment.

Fermi's solution. There is a light cloudy growth in two to three days. The growth is at first white, with a good pellicle. After several weeks a light green color appears, which, however, never becomes distinct. A heavy, stringy, white sediment is formed.

Cohn's solution. There is no growth.

Potato cylinders. A very light colorless growth appears along the streak in approximately twenty-four hours. In some cases the growth is barely visible. The potato cylinders turn somewhat gray.

Cellulose digestion. Fair growth is obtained in the broth, but there is no digestion of cellulose. Three strains were tested, all of which had been in culture for approximately a year.

Starch agar plates (pH 6.6). Plates streaked five or six times, and tested at the end of two, six, and ten days, show no digestion of starch. In determining this, the plates were flooded with a saturated solution of iodine in 50-per-cent alcohol.

Dextrose broth. A cloudy growth occurs in twenty-four hours in the open arm of a fermentation tube. A light acid reaction appears in one to three days, and gradually fades back to the alkaline side. In some cases there is growth in the closed arm of the tube. A pellicle is formed. There is a slight acid production, but no gas.

Saccharose broth. A light cloudy growth occurs in twenty-four hours. A light acid reaction appears in one to two days, and the medium then gradually becomes alkaline. The color reaction is more vivid in test tubes than in fermentation tubes. Growth occurs sometimes, but not always, in the closed arm of a fermentation tube. There is a slight acid production, but no gas.

Lactose broth. A light cloudy growth appears in twenty-four hours. There is no further reaction, and no acid nor gas is produced.

Maltose broth. A light cloudy growth may be noted in twenty-four hours. No acid nor gas is produced.

Indol production. A good cloudy growth appears in trytophane broth in twenty-four hours. Tests made for indol at the end of one and of four days, by the Ehrlich-Böhme method, were negative.

Nitrate reduction. The organism does not reduce nitrates to nitrites when grown on nitrate broth and tested at the end of two, four, and eight days with sulfanilic acid and a-naphthalamine in acetic acid.

Hydrogen-sulfide production. A very light to fair growth is obtained on Wilson's medium, but the reaction is negative. A good growth is obtained in lead-acetate agar. From a negative reaction to a very faint browning occurs along the stab.

Chromogenesis. This organism apparently belongs to the green-fluorescent group. Its ability to produce this pigment, however, is weak. The production of a green color, even by recently isolated strains, has never been noticed on beef-extract agar, bouillon, or gelatin. The pigment is

formed, nevertheless, in Uschinsky's solution, and to a less extent in Fermi's solution. The cultural characteristics of this pathogene agree very closely with those of other members of the green-fluorescent group.

Oxygen relations. The oxygen relationship of this organism varies with the strains tested. Certain strains always appear to grow in the closed arms of fermentation tubes in dextrose and in saccharose broth, while other strains vary in this character. In shake agar cultures in which a beef-extract agar with 1 per cent of dextrose was used (pH 7.0), one strain did not produce colonies more than 3 millimeters below the surface, while in other strains colonies were found as deep as 3.5 centimeters below the surface. One strain isolated from bean pods collected in Switzerland had the largest colonies, and the greatest number of them, from 6 to 7 millimeters below the surface.

Longevity. The pathogene, while it frequently does not make a good growth on the various agars, is a hardy organism and does not readily die in pure culture. Several cultures on beef-extract agar kept at a temperature of 3° to 6° C. lived for fifteen months. At the end of that time, subcultures proved to be very virulent when inoculated into bean seedlings.

Thermal death-point. Test tubes of a uniform thickness, and containing 10 cubic centimeters of a beef-extract broth, were used in these experiments. Two lots of broth were used at various times, having, respectively, a pH value of 6.9 and 7.0. These tubes were immersed in a water bath for ten minutes. The water was kept agitated, and accurate temperature readings were made continuously with a certified thermometer. From a number of such experiments it was determined that the thermal death-point was approximately 49° C.

Brief description

Phytomonas medicaginis var. *phaseolicola* is a non-spore-forming rod with rounded ends, occurring singly or in pairs. The cells measure 1.35 to 3.6μ by 0.9 to 1.2μ. Filaments are often produced. The organism is motile by means of a single polar flagellum. It stains readily with carbol fuchsin, gentian violet, and Loeffler's methylene blue, showing granules which often are bipolar. It is Gram-negative, not acid-fast, and a facultative anaerobe.

The colonies on nutrient agar are white to colorless, slow- to moderate-growing, concentrically ringed, with edges slightly undulate; gelatin colonies are slow-growing, circular, raised, and somewhat wrinkled; agar streaks are of moderate growth, colorless to white, flat, glistening, translucent, and butyrous at first but later brittle; in nutrient broth a cloudy growth occurs within twenty-four hours and a pellicle is formed; litmus milk becomes an intense blue, with no peptonization; a small amount of acid is produced in dextrose and in saccharose broth, but none in lactose

or in maltose broth; no gas is formed in these sugar broths; nitrates are not reduced to nitrites, nor is gas formed in nitrate broth; gelatin is slightly liquefied; starch is not digested; there is no production of indol; hydrogen sulfide is not produced or but slightly; there is a light yellow-green turbid growth in Uschinsky's solution, a slight trace of green in Fermi's solution, and no growth in Cohn's solution; the thermal death-point is approximately 49° C.

The organism is pathogenic on the common bean (*Phaseolus vulgaris*), the sieva bean (*P. lunatus*), and the scarlet runner bean (*P. coccineus*).

A NEW BACTERIAL DISEASE OF THE BEAN, FOUND IN NEW YORK AND NEW JERSEY

(Caused by *Phytomonas vignae* var. *leguminophila* n. var.)

In August, 1926, specimens of diseased leaves of a garden variety (name unknown) of *Phaseolus vulgaris* were received from Dr. C. Chupp. These had been collected in a field in New Jersey where the disease was said to be very severe. The lesions on the leaves were rather unusual in appearance. They were smaller than those spots which one associates with *Phytomonas phaseoli* or *Phyt. medicaginis* var. *phaseolicola*. They were red-brown in color, and when the specimens were received in Ithaca the lesions appeared to be somewhat raised above the surrounding tissue.

In an attempt to isolate a pathogene from the spots, numerous white bacterial colonies appeared in the plates. They were larger and more rapidly growing than *Phyt. medicaginis* var. *phaseolicola*, and on beef-extract agar they produced a green fluorescent pigment. Inoculation experiments were conducted immediately to determine the pathogenicity of the organism for the bean plant. In these first experiments the incubation period was short; infection appeared in two days when seedlings were inoculated by pricking the stem, and the death of the seedling occurred within a week.

In the following year (August, 1927), specimens of diseased bean leaves were again received which had been collected in the same field in New Jersey. Some of the lesions on these leaves were large and of the type usually considered to be caused by *Phyt. phaseoli*. The majority, however, were small, and were similar to those described above. Nevertheless, in no instance was a green fluorescent organism isolated, and in every attempt at isolation, from no matter what type of lesion, *Phyt. phaseoli* appeared in the plates in abundance.

On September 3, 1926, diseased leaves of the yard-long bean (*Vigna sesquipedalis*) were collected in the vegetable-garden plots on the university farm at Ithaca, New York. The lesions were similar in appearance to lesions caused by *Phyt. phaseoli* on leaves of *P. vulgaris*, but were somewhat lighter in color. *Phyt. phaseoli*, however, is not pathogenic to *V. sesquipedalis*. A white organism producing a green fluorescent pigment was

isolated from these lesions, and also was found to be pathogenic to *P. vulgaris*. This strain of the organism, which differed but slightly from that from New Jersey, is referred to in this memoir as the "Q strain."

For use in further study, for inoculation purposes and to determine the cultural characteristics, the two strains of the pathogene were reisolated from artificially inoculated bean plants showing the disease, and were transferred from dilution culture plates.

INOCULATION EXPERIMENTS

On the bean

This disease has not been studied in the field under natural conditions. What is known concerning it has been learned by examination of plants artificially inoculated, and usually under greenhouse conditions. The variety of bean used in the majority of cases was Wells' Red Kidney, a variety which proved to be highly susceptible to the pathogene.

When the organism is introduced into the stem of a bean seedling or a young plant, the disease appears within approximately four days. This is an incubation period which does not differ greatly from those of several of the other bean pathogenes. The mode of attack, however, differs somewhat from that of the other pathogenes with the exception of *Phytomonas viridiflava*. The organism here considered is not a vascular parasite, as is *Phyt. flaccumfaciens*, nor a facultative vascular parasite, as are *Phyt. phaseoli* and *Phyt. medicaginis* var. *phaseolicola*. It does not show a preference for the vascular system, although it sometimes follows the xylem vessels. Consequently, when inoculated into the stem the bacteria seem to invade all tissues. A sunken necrotic lesion ensues, but the epidermis usually remains intact except at the point of inoculation. The plant parts above this lesion die, due to their being cut off from the roots and not necessarily due to the invasion of the organism in these parts. When a seedling wilt results from an artificial inoculation, it is often accompanied by a toppling-over of the plant at the point of inoculation. If not all of the vascular tissue is affected at this point, the plant may recover somewhat.

The disease never appears to be systemic without showing lesions along the stem. Older plants that have become woody are more or less resistant to the pathogene. In such cases the lesion may be on only one side of the stem and the plant will continue to live. In all cases of infection on the stem and the leaves, the organism works rapidly at first, but after a few days the border of the lesion does not extend appreciably. Either the invasive ability of the pathogene diminishes, or a certain resistance is shown in the host. This mode of attack is entirely different from those of *Phyt. phaseoli*, *Phyt. phaseoli* var. *fuscans*, *Phyt. flaccumfaciens*, and *Phyt. medicaginis* var. *phaseolicola*, in all of which the persistency of the pathogene remains fairly consistent.

FIGURE 12. RED KIDNEY BEAN PODS SHOWING RING SPOT LESIONS RESULTING FROM ARTIFICIAL INOCULATIONS WITH PHYTOMONAS VIGNAE VAR. LEGUMINOPHILA

A very characteristic and peculiar lesion occurs on the pods of Wells' Red Kidney. About forty-eight hours after inoculation a ring spot appears, somewhat less than a half inch in diameter and circling the point of inoculation. The color of the ring is at first pale brown, and that of the tissue within is a normal green. This ring spot does not retain its identity long, since the central tissue soon collapses and turns brown. These spots never show a water-soaked condition at any stage, and usually at the end of a week they are dark olive-buff to tawny olive in color.

When bean plants are sprayed with a water suspension of the organism and kept in a moist chamber for twenty-four hours, small spots are produced, but only on the young leaves. These lesions are very small, 3 millimeters in diameter, and under the conditions of observation have never expanded.

The Q strain, besides being a weaker pathogene than the New Jersey strain, never produced the ring spot on the bean pods. Otherwise the symptoms of the two strains are similar.

Cross inoculations

This pathogene, especially the New Jersey strain, has a very wide host range among the leguminous plants. Of twelve species of legumes used in inoculation experiments, eight proved to be susceptible. Experiments were not conducted with any non-leguminous plants. All tests were made in a greenhouse with a fairly constant temperature of approximately 80° F. Inoculations were being made continuously on seedlings of *Phaseolus vulgaris*, to make certain that a virulent culture of the bacterium was being used. When the virulence lagged, reisolations were made.

In all cases, seedlings or young plants of the species being tested were employed. In most cases, inoculations were made also on pods of such species. It was practically impossible, however, to obtain in the greenhouses pods of the scarlet runner bean or of the kudzu vine. The following species proved susceptible to the disease: sieva bean (*P. lunatus*), white-flowering hyacinth bean (*Dolichos lablab*), kudzu vine (*Pueraria hirsuta*), cowpea (*Vigna sinensis*), yard-long bean (*V. sesquipedalis*), soybean (*Glycine max*) variety Wilson, and broad bean (*Vicia faba*). Good infection was obtained on the pods of soybeans, but not on the seedlings.

Negative results occurred when inoculations were made on scarlet runner bean (*P. coccineus*), white-flowering lupine (*Lupinus polyphyllus*), field pea (*Pisum sativum* var. *arvense*), and sweet clover (*Melilotus alba*).

The foregoing results were obtained with the New Jersey strain of this organism. The Q strain caused infection only on the common bean, the kudzu vine, the cowpea, and the yard-long bean. This strain, not only in its cultural reactions but also in its pathogenicity, appeared to be weaker than the other. The type of lesion produced on the above-named

plants, however, appeared to be the same. Considering the number of species, and even of genera, that the New Jersey strain infected, it seems strange that the scarlet runner remained immune. Many and repeated inoculations were made on this species, however, without any positive results.

Inoculations were always made on seedlings or young plants of all the species tested. Infections in all cases were similar to those occurring on the Red Kidney seedlings. The disease appeared, and in several days spread rapidly from the point of inoculation, and then further invasion of the tissue ceased altogether. In a few cases, inoculations were made on the pods of some of the above-named species. This was done by pricking the tissue with a sharp-pointed instrument. The following peculiarities were noted: a more-or-less water-soaked lesion occurred on pods of *Phaseolus lunatus*, while a dry red-brown spot appeared on pods of *Vigna sinensis*; the spots on pods of *Glycine max* (Plate IV, D) were irregular in shape, and dark brown to almost black in color. A ring spot was produced in some cases, but not in all, on pods of *Dolichos lablab*.

One attempt was made to obtain infection by spraying seedlings of the yard-long bean, the soybean, and the lima bean, but with no success.

As stated above, infection was obtained in *Vicia faba*. This is the only organism described in this memoir which attacked this species. The plants which were inoculated were from 9 to 12 inches high, but the stems were still succulent. Black, sunken lesions occurred at the point of injury and extended up and down the stem. Leaves adjacent to the spots withered and turned black, and leaves far above them turned black about the tip. Although none of the plants died, they became stunted and very ragged in appearance.

ETIOLOGY

Morphology

The pathogene, when grown on beef-extract agar (pH 6.9), is a fairly large rod with rounded ends, occurring singly or in pairs. In a few cases the rod is slightly curved. A culture twenty-four hours old, grown at 27° C. on the above medium, with a Congo-red negative stain, had the following dimensions: 2.02μ (1.35 to 3.6μ) by 1.07μ (0.6 to 1.8μ).

The organism stains readily with carbol fuchsin and gentian violet. It is Gram-negative and is not acid-fast. It is actively motile, with one to two polar flagella. Capsules have not been demonstrated; Huntoon's method was employed for this test.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated)

Beef-extract agar slants (pH 7.0). A good growth appears along the streak within twenty-four hours, white to almost colorless. The growth on a

three-days-old culture is filiform, white, translucent, smooth, and glistening. Freshly isolated cultures produce a marked green fluorescent pigment. The ability to produce this pigment diminishes in strains grown long in culture.

Beef-infusion agar slants (pH 7.0). A very good whitish transparent growth occurs along the streak, similar to that on beef-extract agar. A very marked yellow-green fluorescent pigment is produced.

Beef-extract agar plates (pH 7.0). Seven-days-old colonies are white to hyaline, smooth, amorphous, with edges entire. They are butyrous in consistency, and not more than 5 millimeters in diameter. Q strain appears bluish to opalescent by transmitted light. Older colonies may show a zonation.

Beef-extract bouillon (pH 7.0). The broth becomes cloudy in twenty-four hours, and very cloudy within two days. A light flocculation is produced in seventy-two hours, and within a week a whitish granular sediment is formed.

Gelatin stab (pH 6.6). (Temperature 21° C.) Growth appears along the stab within twenty-four hours, with slight liquefaction at the surface. This liquefaction is at first turnip-shaped, but it proceeds rapidly and soon loses any characteristic shape. Complete liquefaction occurs in about ten days. The liquefied gelatin becomes cloudy, and a heavy sediment forms within it which is slightly yellowish. A green fluorescent pigment is produced. With the Q strain, liquefaction proceeds more slowly and is not complete for six weeks.

Milk. Separation begins on the fifth day, and occurs throughout the culture on the sixth day. Further changes are slow, but at the end of six weeks the culture appears as a muddy yellow liquid. With the Q strain, clearing is very slight.

Litmus milk. A slight blue clearing appears at the top of the culture in two days. This clearing continues, and is nearly complete at the end of eight days. Litmus is reduced from the bottom of the tube upward. In eleven days a muddy purple appearance is evident, and at the end of six weeks the culture is muddy brown. The Q strain is much slower in reaction.

Brom cresol purple milk. Procedure is similar to that in litmus milk, with the exception of the color. A clear and purple zone appears at the top and extends throughout the tube. The color is a reddish purple by transmitted light. At the end of six weeks the culture is a fairly clear wine-colored liquid.

Uschinsky's solution. There is a slight clouding in twenty-four hours, and the culture becomes very cloudy, with a pellicle, in forty-eight hours. In one week a light flocculation appears, and in five weeks the culture is turbid, with a very heavy sediment. At the end of the fourth day a light greenish color may be observed, which deepens and appears in

three weeks as an olivine to malachite green. At the end of six weeks the culture is a pale grass green.

The Q strain is white until the tenth day. The color is not likely to become as intense as with the other strain.

Fermi's solution. Slight clouding appears in twenty-four hours, and increases until a good growth is reached in forty-eight hours. A good pellicle is formed by the fourth day. The culture becomes white and milky, and a heavy sediment is formed. No further change occurs in six weeks.

Cohn's solution. There is no growth.

Potato cylinders. At the end of two days the growth is scarcely visible. The medium becomes gray, but there is no further change at the end of seven weeks.

Cellulose digestion. A good growth takes place in broth, but there is no indication of cellulose digestion.

Starch agar plates (pH 6.6). Plates streaked and tested with a saturated solution of iodine in 50-per-cent alcohol at the end of two, six, and ten days show no noticeable digestion.

Dextrose broth. A cloudy growth occurs in the open arm of a fermentation tube in twenty-four hours, and the medium becomes slightly acid. The acidity increases, but by the tenth day a reaction occurs and at the end of sixteen days all cultures appear alkaline. A cloudy growth appears in the closed arm of the fermentation tube. No gas is produced.

Saccharose broth. A light cloudy growth is noticeable in the open arm of a fermentation tube at the end of twenty-four hours. The broth is slightly acid, and reaches its maximum acidity within three days. The acidity is never so pronounced in test tubes as in fermentation tubes, and the broth in the test tubes reverts to the alkaline side very quickly. A cloudy growth occurs in the closed arm. Acid is produced, but no gas.

Lactose broth. A light cloudy growth occurs in the open arm of a fermentation tube in twenty-four hours. No further change in the broth is noted. No acid nor gas is produced.

Maltose broth. A cloudy appearance may be seen in twenty-four hours in the open arm of a fermentation tube, but there is no acid reaction. The culture appears the same at the end of five weeks, except that there is a pale yellow-brown sediment which has formed gradually.

Shake agar culture. In beef-extract agar with 1 per cent of dextrose (pH 7.0), colonies may be observed from 5 to 8 millimeters below the surface at the end of six days. No further colonies appear after that time.

Indol production. The organism produces no indol when grown in tryptophane broth. The Ehrlich-Böhme test was used at the end of one and of four days. Positive results were obtained on this medium with *Escherichia coli.*

Nitrate reduction. There is no reduction of nitrates to nitrites. The organism was grown in nitrate broth, and was tested with sulfanilic acid and a-naphthalamine in acetic acid at the end of two, four, and eight days. Positive results were obtained with *Esch. coli.*

Hydrogen-sulfide production. A good growth is obtained on Wilson's medium, but there is no positive reaction. On lead-acetate agar, at the end of a month there is a very slight browning along the stab.

Chromogenesis. The organism produces a water-soluble green fluorescent pigment on various media. This pigment is the most noticeable in Uschinsky's solution; it appears on beef-extract agar and on beef-infusion agar, but not in Fermi's solution. A recently isolated organism produces the pigment better than do the older strains.

Loss of virulence. When the New Jersey strain of this pathogene was first isolated, its virulence was exceedingly high. When it was inoculated into a bean seedling or a young plant, the disease progressed rapidly and the plant was killed in approximately a week. The invasive ability of the pathogene, however, gradually diminished in pure culture, until at the end of eight or nine months it was able to produce only a small lesion on a bean seedling. Its ability to produce a pod spot was not reduced so greatly. In May, 1927, the organism was inoculated into a pod of the sieva bean (*Phaseolus lunatus*). This pod was kept in the laboratory in a dry condition until October of the same year, when the pathogene was reisolated. It was found then that the organism had regained its former virulence. This process was repeated several times during the winter of 1927–28 with the organism, so that its virulence might be retained and it might be used in conducting inoculation experiments.

Thermal death-point. Test tubes of a uniform thinness, and containing approximately 10 cubic centimeters of beef-extract broth (pH 7.1), were used. Two large loops full of a broth culture twenty-four hours old were added to the tubes, and the tubes were immersed in a water bath for ten minutes. The water was kept agitated, and accurate temperature readings were made continually with a certified thermometer graded in tenths of degrees. The thermal death-point appeared to be approximately 49° C.

Nomenclature

It has been pointed out by the writer in a previous article (Burkholder, 1930) that the green fluorescent bacteria which cause plant diseases are a rather closely related group. It is not surprising, then, to find that the pathogene under consideration here agrees so closely in its cultural reactions with one of the nineteen or more previously described species. The species to which it is very closely allied is *Phytomonas vignae,* which also attacks certain leguminous plants. There are several distinct differences between the two pathogenes, however, both in cultural characteristics and in host

range, which would keep them from being considered identical. *Phyt. vignae* is described as producing an abundant growth on potato cylinders and causing no change in the color of this medium. In Fermi's solution it produces a green-yellow pigment. The organism dealt with here will barely grow on potato, and causes the medium to turn gray; and in Fermi's solution a white, milky growth is produced. The differences are not great, but they do show that the two pathogenes are not the same.

There is a more pronounced difference in their pathogenicity, however. With *Phyt. vignae*, Gardner and Kendrick (1925) obtained negative results on garden beans, soybeans, and broad beans. Infection was produced in all of these species by the organism under consideration here. So susceptible are the garden bean and also the broad bean, that there could be no mistake concerning this character. Consequently it is felt that this is a distinct pathogene, although one closely related to the cowpea organism. For these reasons it is held as a variety of *Phyt. vignae*, and because of its broad host range is given the name *Phytomonas vignae* var. *leguminophila* n. var.

Brief description

Phytomonas vignae var. *leguminophila* is a non-spore-forming rod with rounded ends, occurring singly or in pairs. The cells measure 1.35 to 3.6μ by 0.6 to 1.8μ. The organism is motile by means of one to two polar flagella. It is Gram-negative, not acid-fast, and a facultative anaerobe.

The colonies on beef-extract agar (pH 7.0) are round, white to hyaline, smooth and amorphous, with the edges entire, producing a water-soluble green fluorescent pigment; nutrient broth is cloudy within twenty-four hours; gelatin liquefaction is fairly rapid; milk turns alkaline, clears, and later becomes muddy; litmus is reduced; a green fluorescent pigment appears in Uschinsky's solution; there is a white milky growth in Fermi's solution; starch is not digested; growth is very slight on potato; acid is produced in dextrose and in saccharose broth, but none in lactose or in maltose broth; gas is not produced in these sugar broths; indol is not produced; nitrates are not reduced to nitrites; hydrogen sulfide is not produced or but slightly; the thermal death-point is approximately 49° C.

The organism is pathogenic on the common bean (*Phaseolus vulgaris*) and on a number of closely related species.

A HITHERTO UNREPORTED BACTERIAL DISEASE OF THE BEAN, FROM EUROPE

(Caused by *Phytomonas viridiflava* n. sp.)

In August, 1927, green-bean pods were observed in a market in Lucerne, Switzerland, showing lesions which, from appearances, were due to a bacterial invasion. The lesions were rather reddish brown in color, and

were circular in outline except where anastomosing occurred. Judging from the pod, the bean was of a Refugee green-pod type. A number of these pods were collected and dried, and later, in October of the same year, an attempt was made to isolate an organism. In making these isolations, small parts of the lesions were macerated in sterile water, and dilution plates were poured from these. Bacterial colonies, white to cream yellow in color and producing a green fluorescent pigment, appeared in profusion in all the plates. In the following experiments, three different strains isolated from three different pods were used. The strains were, however, practically identical.

In testing the pathogenicity of these organisms, bean seedlings were inoculated at the cotyledon node by pricking and introducing the bacteria at the same time. Two days after the seedlings were inoculated, infection occurred, and within four days the plants toppled over and were dying. The virulence of the organism was extremely high. In no other case under observation had a pathogene acted with such rapidity. For further work with the pathogene, reisolations were made of the three strains and the pathogenicity of each was again tested.

INOCULATION EXPERIMENTS

On the bean

The disease produced by this pathogene has never been studied nor observed in the field on the living host. After the first specimens were collected in the market of Lucerne, all observations and experiments were conducted on plants artificially inoculated. This was done in a greenhouse under constant temperature control of approximately 80° F. The variety of bean used was Wells' Red Kidney. The striking peculiarities of the disease were observed under these conditions, and they are such as to set it apart from any of the other bacterial blights of the bean.

In general, this Swiss organism appears to be the most virulent of the bacterial pathogenes treated in this memoir. At least, its incubation period is the shortest, and its invasive ability for the first few days is the greatest. When inoculated into a young plant, the organism spreads rapidly and invades all the tissues within its path, killing them and producing a necrotic lesion. Infection appears in about two days. The extent of the invasion, however, is reached possibly by the fifth day or in a little less than a week. Then the invasive ability of the pathogene apparently ceases altogether. Whether this is owing to the organism or is a host reaction has not been determined.

The lesion on the main stem of a young plant will extend probably less than an inch above and below the point of inoculation. This depends to a certain extent on the age and the succulency of the tissues involved. With the killing of the cells throughout the main stem, all parts of the

plant above a lesion will die. The collapse in most cases is sudden and complete. This death of the upper part of the plant is in no respect similar to the wilt due to the xylem invasion by many of the other bacterial bean pathogenes.

Below the lesion the tissue of the plant remains healthy, and if a toxin is formed by the bacteria it is not one which rapidly spreads ahead of the invaded cells, as is found in the case of *Phytomonas medicaginis* var. *phaseolicola*. There is no gradation between the dead tissue and the healthy, as may be found in many necrotic diseases; instead, a sharp line of demarcation exists between the two tissues, and this remains stationary since the disease does not move downward. The healthy tissue, however, begins to form a gall, which breaks out from underneath the epidermis and often becomes twice the diameter of the original stem (figure 13).

It is reasonable to expect that the host plant would react by the formation of such a gall merely because of the accumulation of food material at this point. In other words, the reaction may be due to the mechanical injury of the disease. *Phyt. vignae* var. *leguminophila* may cause a similar type of necrotic lesion on the bean plant, but in no case has gall formation been observed. This would appear to indicate that the pathogene under consideration played some part in the formation of the gall. This indication was strengthened further when it was observed that hypertrophy occurred about relatively small lesions caused by the Swiss organism on the stems of sieva beans. It must be admitted, however, that no careful histological studies or attempts have been made to ascertain whether bacteria are present in the galls or whether the bacteria produce a substance which would stimulate gall formation.

The action of the pathogene on the bean pod is somewhat different from its action on the stem. On the pod no hypertrophy has been observed, and, while the bacteria move rapidly in the tissue, there is not that flash of invasion followed by sudden ceasing of growth which is found in the stem tissue. If inoculations are made by injuring the surface of the pod, the pathogene progresses rapidly from the point of inoculation and produces a spot which is slightly sunken and from pecan brown to walnut brown in color. There is no green, water-soaked appearance, as is found in the early stages of pod lesions produced by *Phyt. phaseoli*, its variety *fuscans*, and *Phyt. medicaginis* var. *phaseolicola*, or by the suture lesions produced by *Phyt. flaccumfaciens*. The spot has more the appearance of a late stage of a spot produced by *Phyt. vignae* var. *leguminophila*. In certain instances when the injury to the pod tissue has been deep, the bacteria develop rapidly and ooze out with the sap in small, reddish brown drops.

In a few cases, inoculation experiments were conducted on seedlings and on pods without injuring the plants. The seedlings were sprayed with a suspension of the bacteria in water, and a similar suspension was painted

FIGURES 13. BEAN PLANTS THAT HAVE BEEN INOCULATED WITH PHYTOMONAS VIRIDIFLAVA, SHOWING GALL FORMATION BELOW THE DEAD TISSUES

on the pods with a camel's-hair brush. In no case was infection produced. The experiments were too few in number, however, and were not conducted under sufficiently varied conditions, to permit of drawing any definite conclusions.

Cross inoculations

To determine the host range of this green-fluorescent pathogene, and its pathogenic relationships to others of its type, inoculation experiments were conducted on a dozen species of legumes besides the common bean (*Phaseolus vulgaris*). The experiments were made in a greenhouse at a temperature of approximately 80° F. Seedlings were used in all cases, and in a few instances green pods of certain species were inoculated. Inoculations were made by pricking the plants with a sharp-pointed instrument and introducing the inoculum at the same time.

Good infection was obtained on seedlings of the following species: scarlet runner bean (*P. coccineus*), sieva bean (*P. lunatus*), white-flowering hyacinth bean (*Dolichos lablab*), cowpea (*Vigna sinensis*), and kudzu vine (*Pueraria hirsuta*).

While the results were negative on seedlings of the soybean (*Glycine max*), a high degree of infection was obtained on the pods of this species when these were injured (Plate IV, E). The pod spots were angular in shape but very definite in outline, and were light brown in color with usually a dark border. The variety of soybean used was Wilson. Good pod infection was obtained also on the sieva bean.

Negative results were obtained on seedlings of the following species: white-flowering lupine (*Lupinus polyphyllus*), yard-long bean (*Vigna sesquipedalis*), broad bean (*Vicia faba*), field pea (*Pisum sativum* var. *arvense*), and sweet clover (*Melilotus alba*). Owing to the fact that in culture this pathogene rapidly loses its virulence, and even its pathogenicity, less satisfaction is felt with this list of plants giving negative results than with any other list in the memoir. It is true that more than one set of inoculations were made on the species listed above, and in all sets of experiments certain species in the susceptible list succumbed, showing that the culture used was composed of virulent organisms. Nevertheless, because of the inconsistency with which the pathogene sometimes refused to infect susceptible plants, the negative results were regarded with some uncertainty.

ETIOLOGY

Morphology

From a culture twenty-four hours old on beef-extract agar (pH 6.9), grown at a temperature of 27° C., individual cells are large rods with rounded ends. Considerable variation, however, occurs among them. They measure 2.13μ (1.35 to 3.6μ) by 0.81μ (0.6 to 1.2μ).

The organism stains well with carbol fuchsin and gentian violet. Granules, both central and polar, have been observed. The pathogene is Gram-negative and not acid-fast. Capsules have not been demonstrated. All cultures on agar showed an actively motile organism, which, when stained with Loeffler's flagella stain, was found to have one to two polar flagella.

Cultural characteristics

(All cultures incubated at 27° C. unless otherwise stated)

Beef-extract agar slants (pH 7.0). Growth appears along the streak within twenty-four hours. At the end of three days there is a very good growth, filiform, smooth, glistening, and translucent. The bacteria become cream-colored to definite yellow with age. Cultures are butyrous in consistency. A slight green fluorescent pigment is dissolved in the medium.

Beef-infusion agar slants (pH 7.0). A very good growth occurs, creamy to yellow but otherwise similar in appearance to the growth on beef-extract agar. A very marked yellowish green fluorescent pigment is produced, which is dissolved in the medium.

Beef-extract agar plates (pH 7.0). At the end of a week the colonies are approximately 5 to 7 millimeters in diameter, smooth, naples yellow to cream color, with edges undulating. A weak green fluorescent pigment is dissolved in the medium.

Beef-extract bouillon (pH 7.0). The medium becomes cloudy in twenty-four hours, and very cloudy in forty-eight hours. A light flocculation occurs throughout the tube, and in some cases a delicate pellicle is formed. A sediment, white and stringy, gradually accumulates.

Gelatin stab (pH 6.6). (Temperature 21° C.) Growth appears along the stab within twenty-four hours, with slight liquefaction at the surface. Liquefaction proceeds rapidly, being complete in seven to ten days. It is turnip-shaped at first, and later stratiform. A heavy white sediment is formed, and the liquefied medium becomes cloudy at the surface.

Milk. Clearing of the milk begins at the surface on the fourth to the sixth day, and extends throughout the tube by the end of a week. With age the medium becomes a brownish, muddy liquid, with a slight sediment.

Litmus milk. At the end of three days a clearing begins at the surface, and this gradually extends throughout the medium. Litmus turns slightly blue and is reduced. The milk then becomes muddy, often with a purple tinge.

Brom cresol purple milk. On the third day the medium has become purple, and a light clearing has begun at the surface of the milk which later extends throughout the tube. Within a week the milk is a clear red-purple liquid by transmitted light. At the end of six weeks little further change has occurred.

Uschinsky's solution. The solution is slightly cloudy in twenty-four hours, and very cloudy in several days. A green fluorescent pigment is formed, which is very pale at first and changes through various shades of green until at the end of a week it is an olive-yellow. An orange-yellow sediment is formed.

Fermi's solution. A slight clouding appears in twenty-four hours, and in four days there is a very good growth. In a week a pellicle appears which in some cases becomes very heavy and falls to the bottom of the tube. A green fluorescent pigment appears after several days to a week, and at the end of six weeks this is a water green in color.

Cohn's solution. There is no growth.

Potato cylinders. At the end of two days there is a very slight growth on the surface of the potato, but no further signs are noticeable. The medium turns slightly gray.

Cellulose digestion. The organism grows well in the broth, but there is no indication that the cellulose is attacked.

Starch agar plates (pH 6.6). Plates streaked five or six times, and tested two, six, and ten days after inoculation with a saturated solution of iodine in 50-per-cent alcohol, show no digestion of starch.

Dextrose broth. The broth in the open arm of a fermentation tube becomes cloudy in twenty-four hours. It is at this time slightly acid, and within four days it is distinctly acid, after which it gradually returns to the alkaline side. A yellow-orange to brown pigment is formed in this medium, which is very marked in one strain. In most cases there appears to be a fair growth in the closed arm of the fermentation tube. No gas is produced.

Saccharose broth. A light clouding appears in twenty-four hours in the open arm of a fermentation tube. Growth is better on the second day, but there is no growth in the closed arm of the tube. No acid nor gas is produced.

Lactose broth. A light cloudy growth appears in twenty-four hours and reaches its optimum in four days. No acid nor gas is produced.

Maltose broth. A cloudy growth appears in twenty-four hours. No acid nor gas is produced.

Shake agar culture. In beef-extract agar with 1 per cent of glucose (pH 7.0), colonies are not found lower than 2 millimeters below the surface.

Indol production. The bacteria grow well in trytophane broth, and tests for indol at the end of one and of four days with the Ehrlich-Böhme method have been negative.

Nitrate reduction. Good growth occurs in nitrate broth, and tests for nitrites made at the end of two, four, and eight days with sulfanilic acid and a-napthalamine in acetic acid show no presence of nitrites. A positive test was obtained on the above medium with *Escherichia coli.*

Hydrogen-sulfide production. Growth appears on Wilson's medium within three or four days, but there is no positive reaction. On lead-acetate agar there is a faint browning along the stab. The bacterial mass at the surface appears rather blue-green in color.

Chromogenesis. Several kinds of pigments are produced by this organism on various media. A green fluorescent pigment, which is water-soluble,

is formed when the pathogene is grown on beef-extract or beef-infusion agar, and is very conspicuous in Fermi's and in Uschinsky's solution. Also, on the agars the bacteria cells may be of a creamy color in young cultures to a naples yellow in older ones, and in many solutions and broths a yellowish sediment is formed. In dextrose broth an orange-yellow pigment colors the entire medium, and one strain of the pathogene produced it to such an extent that it was difficult to detect the Andrade's indicator in the medium.

Virulence. A loss of virulence, and even of pathogenicity, of the organism occurs in pure culture. This was first noted when a strain isolated in October, and re-isolated on November 10 of the same year, showed little invasive ability on the 1st of January. For making inoculation experiments, and especially cross inoculations, re-isolations were made about once a month. In this way the virulence of the organism was kept from waning.

Thermal death-point. Test tubes of a uniform thickness, and containing 10 cubic centimeters of beef-extract broth (pH 7.1), were used in this experiment. To these tubes were transferred two large loops full of a broth culture twenty-four hours old, and the tubes were immersed in a water bath for ten minutes. The water was kept agitated and accurate temperature readings were made continuously. The thermal death-point appeared to vary somewhat with the strain used, from 48° to 50° C.

Nomenclature

The pathogene under consideration belongs to that group of bacteria which produces a water-soluble green fluorescent pigment, and consequently it is more or less related to all the species of that group. As far as known, however, its primary characteristics do not entirely agree with those of any of the previously described forms. For this reason, and from the fact that the disease which it produces on the common bean (*Phaseolus vulgaris*) is very characteristic, it is here considered as a new species and the name *Phytomonas viridiflava* n. sp. is given to it.

Brief description

Phytomonas viridiflava is a non-spore-forming rod with rounded ends, occurring singly or in pairs. The average size of the cells is 2.13μ by 0.81μ. The organism is motile by means of one to two polar flagella. It is Gram-negative, not acid-fast, and a facultative anaerobe. Capsules have not been demonstrated.

Growth on beef-extract agar streaks is good to abundant, filiform, smooth, glistening, and translucent; the bacteria become naples yellow with age; a green fluorescent pigment is formed on agar and on various other media; bouillon becomes cloudy, with a light flocculation; liquefaction of gelatin is rapid; litmus milk is cleared, and the litmus turns slightly

blue and is reduced; a green fluorescent growth appears in Uschinsky's and in Fermi's solutions; there is no growth in Cohn's solution; growth is slight on potato; there is no digestion of starch in starch agar; acid is produced in dextrose broth, but not in saccharose, lactose, or maltose broth; no gas is produced in any of these sugar broths; indol is not produced; nitrates are not reduced to nitrites; hydrogen-sulfide production, if any, is slight; the thermal death-point varies from 48° to 50° C.

The organism is pathogenic on the bean (*Phaseolus vulgaris*) and on a number of related leguminous plants.

ANOTHER STRAIN OF PHYTOMONAS VIRIDIFLAVA

Another strain of *Phytomonas viridiflava* was obtained from diseased specimens of bean leaves collected in England. This strain varied in a few respects from the one obtained in Switzerland, but the difference consisted primarily in the English strain being a weaker parasite. Since it has been shown that the Swiss organism loses its virulence, and even its pathogenicity, in pure culture, virulence is scarcely a characteristic to be considered seriously in this species, especially taxonomically. Certain interesting points of pathogenicity, however, are considered worthy of reporting.

The diseased leaves from which the pathogene was isolated were of the scarlet runner type, and the spots observed on them were, in most cases, small. The largest and most conspicuous of these lesions are shown in figure 14. The leaves were collected in a garden near Canterbury, England, in September of 1927, and the isolation of the pathogene was made a month later. The cultural characteristics and the physiological reactions of the English strain were identical with those of the Swiss strain. This was a sufficient reason for considering them of the same species.

The peculiarities of the English strain of *Phyt. viridiflava* became noticeable, however, when attempts were made to infect with it the bean and related species. When inoculations were made on the seedlings of bean plants, infection was not obtained even though the stems of the plants were injured. The bacteria were unable to establish themselves in the tender young tissue of the growing stem, and the mechanical injury made on the plant quickly healed. Small lesions, nevertheless, were produced on the leaves of several species of plants, including *Phaseolus vulgaris* and *P. coccineus*. In these cases the leaves had been punctured with a needle at the time when the inoculum was applied. The spots always remained small, but on miscropical examination it was found that the bacteria were in abundance in the dead and dying cells. This showed that the organism was pathogenic, but its virulence appeared to be low.

Inoculation experiments were then made on the pods of the common bean and a number of related species. Here the results were different, since this tissue proved to be more susceptible to the pathogene. The

FIGURE 14. LEAFLET OF A SCARLET RUNNER BEAN PLANT FROM WHICH THE ENGLISH STRAIN OF PHYTOMONAS VIRIDIFLAVA WAS ISOLATED

spots that developed on the inoculated pods were identical with those produced by the Swiss strain, and the host range was similar. Pods of all the species listed in cross inoculations with *Phyt. viridiflava* were used in inoculation experiments with the English strain, with the exception of *Vicia faba* and *Pueraria hirsuta*. These species did not set pods under the conditions maintained in the greenhouse.

In conducting inoculation experiments on pods of various species, a further peculiarity of the organism and its relation to the host was observed. When very young pods were used in the experiments, no infection was obtained; but extensive infection occurred on maturing pods. When such pathogenes as *Phyt. phaseoli* and *Phyt. medicaginis* var. *phaseolicola* are used in inoculation experiments, almost the reverse of this is true, and it is the case also with the fungous pathogenes of the bean. The susceptible stage in the pod cannot be coupled with a dying of the tissue, since the pod is susceptible for a considerable time before it shows signs of ripening and while it is still in a green and apparently healthy condition. The very young, rapidly growing pods, however, are resistant to the pathogene. This phenomenon is demonstrated readily on such species as *Phaseolus coccineus* and *Vigna sinensis*. It probably is found also with other species, but it was with these two legumes that repeated inoculations and observations were made on young and on maturing pods.

This host relationship probably is tied up with the low virulence of the pathogene, and a somewhat similar case might be cited here. It has been observed with *Phyt. phaseoli* and *Phyt. vignae* var. *leguminophila* that when grown for some time in pure culture they lose their ability to infect the stem of young plants, to produce either a vascular infection or a definite lesion, as is the custom with each organism. Nevertheless, they still retain their ability to infect bean pods. Experiments have never been conducted, however, to determine their relative invasive ability on the pods in various stages of maturity.

DIFFERENTIATION OF THE BACTERIAL DISEASES OF THE BEAN

The differentiation of the various bacterial diseases of the bean is of no consequence to the average grower. The injuries caused by each disease are somewhat similar in appearance, and the sum total is of considerable economic importance. That much the grower appreciates, and a simple method with which to combat these diseases is all that he asks. For the plant pathologist, however, the ability to separate the diseases may be of considerable importance. Aside from the investigation of the diseases with a view to their control, the desirability of determining their distribution and the severity of their epiphytotics in various localities is well understood. In endeavoring to distinguish the various diseases, the determination of the etiological agent is always the final issue. Nevertheless, with these diseases the isolation of the bacteria and their identification

in pure culture is not always possible, and in the majority of cases is not practicable. Too much time and labor would be consumed. If the diseases could be distinguished one from another in the field on a symptom basis, the problem would be greatly simplified. The extent to which the symptoms could be used with certainty should be carefully examined and set forth, together with their limitations. This is attempted in the following discussion.

It should be taken into consideration also that two or more of these diseases may appear on the same plant. In New York, *Phytomonas phaseoli*, *Phyt. flaccumfaciens*, and *Phyt. medicaginis* var. *phaseolicola* sometimes intermingle in the field, and various combinations of these organisms have been isolated from the same diseased individual. Under such conditions a complication of symptoms is set up which is difficult to untangle. Such cases, however, are more likely to be individual, and do not often occur in a bean field.

SYMPTOM BASIS

In the foregoing descriptions of the various bacterial diseases, the most striking and characteristic symptoms of each disease have been pointed out and discussed. In the main these symptoms have been observed in the field on the Red Kidney variety and have been produced on this variety in the greenhouse for purposes of study. To a less degree they have been observed on such common bean varieties as White Marrow, Pea, Refugee, and a few other types found growing in the fields and gardens of New York. More than a hundred varieties of *Phaseolus vulgaris* may be obtained on the market which differ greatly in many of their characters from the Red Kidney bean or the other varieties mentioned above. It is not inconceivable, then, that the symptoms of the bacterial diseases may vary considerably with the variety of beans affected. For this reason it is considered best not to lay too much emphasis on individual symptoms on unknown varieties when endeavoring to separate these bacterial diseases in the field. The Red Kidney and Marrow beans as they occur in New York are the types that are under discussion here.

In considering these symptoms for use as diagnostic characters, certain limitations should be noted. The chief of these limitations arises through the fact that a pathogene may not always produce the same type of symptoms on the bean plant. The disease-producing organism may cause a distinct type of lesion on the leaf, the pod, and other plant parts, but this will not hold in 100 per cent of the cases. Certain anomalies at times are found to exist. These anomalies are seldom to be met with in the greenhouse, where conditions are fairly well controlled, but are found more often in the field, where the environmental factors surrounding the life of a diseased plant can only be guessed at. The causes of these variations in types of lesions may be due primarily to

ecological factors. Temperature and moisture, in some instances, appear to play an important part. In other cases the origin of the lesion, whether exogenous or endogenous, may be of significance. The possibility of there being races of the pathogene with different symptom-producing abilities should not be ruled out of consideration, although such cases have not been proved.

Certain general aspects of the diseases which might prove to be of some diagnostic value should be considered first. As already stated, the diseases caused by *Phytomonas phaseoli* and *Phyt. flaccumfaciens* appear to be favored by hot weather, while the amount of rainfall apparently is not an important factor. On the other hand, *Phyt. medicaginis* var. *phaseolicola* occurs with greater severity in cool seasons. This disease at such times may spread rapidly through a field and work with great speed in the individual plant. In New York no other bacterial disease has caused so much damage, and by the very severity of its action one may almost determine the causal organism.

In considering the individual symptoms of the diseases on the plant as of diagnostic value, they are merely pointed out in this discussion, since they have been described in some detail earlier in the memoir. The most noticeable symptoms of these diseases occur on the leaf, and it is here that most of the peculiarities and characteristic lesions are to be found. The halo-blight leaf spot (figure 6) caused by *Phyt. medicaginis* var. *phaseolicola* is a symptom which can scarcely be mistaken or confused with other leaf spots. This organism, however, does not always produce this type of lesion, and in New York, during the hot months, the common symptom is the small angular spot which occurs in such great numbers over the leaf (figure 7). *Phyt. phaseoli*, however, has been known to produce a spot similar to this latter one, and so this organism should always be kept in mind. In plants systemically infected, certain leaf symptoms indicate when *Phyt. medicaginis* var. *phaseolicola* is the causative agent. These are the mosaic appearance of the upper leaves, and, in severe cases, the dwarfed and yellowish crinkled condition of these leaves. However, in the absence of these symptoms, even though the vascular tissue is affected it is not safe to assume that the disease is caused by some other vascular-invading organism. It should be mentioned here, too, that the mosaic symptoms on the leaves have been produced in the greenhouse on Red Kidney beans artificially inoculated with *Phyt. flaccumfaciens*. While this experiment has been repeated a number of times in the greenhouse, the symptom has never been observed in the field due to this latter organism.

Phyt. phaseoli produces, on the leaf, brown necrotic lesions of varying sizes, with yellow borders (figure 1). These spots may enlarge and cover most of the leaf tissue. The lesion illustrated in figure 8, however, caused by *Phyt. medicaginis* var. *phaseolicola*, is not unlike this type of leaf spot.

Infected leaves of beans have been received from Bermuda, and also from New Jersey, with small spots, reddish brown in appearance and somewhat raised above the surface of healthy tissue (Plate II). Pure cultures of *Phyt. phaseoli* only were isolated from such lesions; nevertheless, *Phyt. vignae* var. *leguminophila* also produces this type of lesion on bean leaves, and so it can scarcely be considered a good diagnostic character.

Phyt. flaccumfaciens, as a rule, wilts the entire leaf or leaflet of the bean, but in some instances it has been known to wilt only a part of the leaf, as in figure 5, which shows a rather large necrotic spot. A similar condition may be produced by *Phyt. phaseoli*, if the necrotic area is of endogenous origin.

On the pod, *Phyt. viridiflava* and *Phyt. vignae* var. *leguminophila* produce spots which from the beginning of their formation are brownish and often dry and necrotic. The other bacteria produce spots which are at first deep green and water-soaked in appearance. The water-soaked condition of the lesion may persist, or under certain circumstances the spots may dry down to reddish sunken areas. Over the pod spots produced by *Phyt. phaseoli* a yellowish crustation may sometimes be observed, while a silvery crustation may be produced by *Phyt. medicaginis* var. *phaseolicola*. This crustation is very delicate and is readily washed off by rains, and therefore it is of very little use in differentiating the two diseases. *Phyt. flaccumfaciens*, as has been pointed out, produces on the green pod only water-soaked lesions along the suture, which are of endogenous origin. This symptom, however, can be duplicated by *Phyt. phaseoli*, *Phyt. phaseoli* var. *fuscans*, and *Phyt. medicaginis* var. *phaseolicola*, and consequently it is not a character to be used without discrimination.

On the stem, *Phyt. phaseoli* produces reddish-colored lesions which extend longitudinally with the stem. A stem girdling at the nodes also is produced, and probably in the majority of cases this arises from an infected xylem. *Phyt. flaccumfaciens* produces only the stem girdle. A very striking peculiarity of *Phyt. medicaginis* var. *phaseolicola* is the formation of the bacterial ooze, or exudate, on the stem lesions (figure 10). No other bacterial disease of the bean has been observed to do this, and therefore this symptom should be looked for in endeavoring to determine the disease under observation. When *Phyt. viridiflava* is inoculated into the stem of a growing bean plant, gall formation, as a rule, occurs. This differentiates the lesion from those due to *Phyt. vignae* var. *leguminophila*.

When a systemic infection occurs in a plant, and a wilt results with no definite stem, leaf, or pod lesions, the indications are that the invasive organism is *Phyt. flaccumfaciens*. This pathogene is almost an obligative vascular parasite, and when it is found in other tissues, as in the pod lesion along the suture, it appears to be more of an overflow from the

xylem tissue. While *Phyt. phaseoli*, *Phyt. medicaginis* var. *phaseolicola*, and to a less extent *Phyt. phaseoli* var. *fuscans*, prefer the xylem and consequently also cause a wilt, these pathogenes are in no way limited to this tissue. They may cause infection in almost any part of the plant. On the other hand, *Phyt. viridiflava*, and to a less extent *Phyt. vignae* var. *leguminophila*, appear to show no preference for the vascular tissue. When they cause a wilt it is due to the invasion of most of the tissues of the stem, including the vascular tissue, and wilting and death of the plant parts above occur as a natural sequence.

In summing up the information, one can only conclude that the best and surest way to diagnose one of these bacterial diseases in the field is to consider the sum total of all the symptoms displayed, and arrive at a decision from them. Even then one cannot always be correct. In table 1, descriptions of the various characteristic symptoms of these diseases have been brought together and arranged to facilitate diagnosis.

TABLE 1. MAJOR SYMPTOMS PRODUCED BY SIX DIFFERENT BACTERIAL PATHOGENES ON THE BEAN (PHASEOLUS VULGARIS)

Pathogene	Wilt	Stem lesions	Leaf lesions	Pod lesions
Phytomonas phaseoli	Result of a vascular invasion	Reddish lesions longitudinal with stem, also stem girdle	Definite necrotic lesions of varying size with yellowish border, Several other types of lesions	Lesions at first water-soaked, angular, anastomosing, drying into reddish sunken areas; lesions along suture of endogenous origin
Phyt. phaseoli var. *fuscans*	Same as with *Phyt. phaseoli*	Same as with *Phyt. phaseoli*, but lesions are inclined to split stem	Same as with *Phyt. phaseoli*	Same as with *Phyt. phaseoli*
Phyt. flaccumfaciens	Result of a vascular invasion. A major symptom	Stem girdle	A part of the leaf, but more often the entire leaf, may wilt	Along suture only, and of endogenous origin; lesions at first water-soaked and green
Phyt. medicaginis var. *phaseolicola*	Result of a vascular invasion	Lesions longitudinal with stem; drops of bacterial exudate sometimes appear	Definite necrotic lesions with light-colored halo; small angular spots; mosaic and yellow symptoms due to systemic infection	Similar to those produced by *Phyt. phaseoli*; a silvery crustation sometimes appears over lesion
Phyt. vignae var, *leguminophila*	Necrotic lesions killing stem, resulting in wilting and death of parts above	Sunken longitudinal lesion which involves all stem tissue	Definite, reddish brown, rather small lesions; wilted parts above stem lesions	Brownish, dry lesions, never water-soaked; sometimes a ring spot
Phyt. viridiflava..	Definite necrotic lesion killing stem, resulting in wilting and death of parts above	Definite lesions involving all tissue; gall production	(?)	Reddish brown spots, never water-soaked

ETIOLOGICAL BASIS

When it is desirable to be very positive concerning the etiological agent of a bacterial disease of the bean, the isolation and determination of the pathogene is essential. This is not a difficult task, since in certain media the bacteria show considerable differences. Little need be said here as to the technic of isolations. All the organisms grow well on beef extract agar or on potato agar. In the lesions on the plants, the bacteria may be found in abundance and in most instances apparently inhibiting all secondary invaders. At least, by following the ordinary precautions only a few contaminating colonies will appear on the plates. Dilution cultures are usually more satisfactory than tissue plantings.

The morphology of the respective pathogenes is not sufficiently characteristic to be of much help in diagnostic work. One must rely on the cultural reactions. In table 2 a few reactions are given which will suf-

TABLE 2. MAJOR DIFFERENCES IN THE CULTURAL REACTIONS OF SIX DIFFERENT BACTERIAL PATHOGENES OF THE BEAN (PHASEOLUS VULGARIS)

Pathogene	Pigment on beef-extract agar	Gram stain	Sugar fermentation			Litmus milk	Potato cylinders	Fermi's solution
			Dextrose	Saccharose	Lactose			
Phytomonas phaseoli	Bacterial cells a distinct yellow	–	–	–	–	Alkaline, clearing; formation of tyrosine crystals in abundance	Bacteria and potato cylinders become a slimy mass	No growth (?)
Phyt. phaseoli var. *fuscans*	Bacterial cells a distinct yellow; medium becomes brown	–	–	–	–	Alkaline, clearing; liquid turns brown	Bacteria and potato cylinders become a brown, slimy mass	No growth (?)
Phyt. flaccumfaciens	Bacterial cells pale yellow	+	Acid	Acid	Acid	Acid, soft curd	None to fair growth; no change in medium	No growth (?)
Phyt. medicaginis var. *phaseolicola*	Bacterial cells transparent to white	–	Acid slight	Acid slight	–	Alkaline, no clearing	Scant growth	Very pale green fluorescent growth
Phyt. vignae var. *leguminophila*	Bacterial cells transparent to white; water-soluble green fluorescent pigment	–	Acid	Acid	–	Alkaline, rapid clearing	Scant growth	Milky white growth
Phyt. viridiflava	Bacterial cells creamy to yellow; water-soluble green fluorescent pigment	–	Acid	–	–	Alkaline, rapid clearing	Scant growth	Good green fluorescent growth

ficiently differentiate all of the organisms. The complete descriptions on preceding pages of the memoir should be consulted also. The color characters may be a little difficult to use, with the exception of the deep

yellow of *Phytomonas phaseoli* and the browning of the media due to the action of *Phyt. phaseoli* var. *fuscans*. The yellow pigment of *Phyt. flaccumfaciens* may be so faint at times as to be scarcely visible, as on agars containing dextrose, and at other times or with other strains it may be as deep a yellow as in *Phyt. phaseoli*. The yellowish cream color of *Phyt. viridiflava* often is not observed. Older cultures show it better than do young cultures, and the sugar broths (dextrose and saccharose) bring out the color as a distinct orange, which may be confused with an indicator if such is present in the medium. The water-soluble green fluorescent pigment appearing on some media is a variable character, but *Phyt. vignae* var. *leguminophila* and *Phyt. viridiflava* have always produced this pigment on beef-extract agar (Bacto-peptone and Armour's beef extract). *Phyt. medicaginis* var. *phaseolicola*, while belonging to the green-fluorescent group, never produces the pigment on beef-extract agar. On the whole, however, it appears safer to base differences on physiological reactions in the media than on pictorial descriptions.

In a previous article (Burkholder, 1930), the writer pointed out that the genus Phytomonas is composed of at least three, and perhaps four, rather distinct groups of bacteria. It is interesting to note that each of the various bacterial pathogenes of the bean plant fall into one of the three distinct groups. *Phyt. phaseoli* and its variety *fuscans* belong to the *Campestre* group; *Phyt. flaccumfaciens* belongs to the *Stewarti* group, which contains the large proportion of Gram-positive and non-motile species; and the remaining organisms, *Phyt. medicaginis* var. *phaseolicola*, *Phyt. vignae* var. *leguminophila*, and *Phyt. viridiflava*, are green-fluorescent forms. While these green-fluorescent bacteria constitute a rather well-defined group of pathogenes, the individual members within are not clear-cut. Many of the pathogenes vary only slightly from the others, both in pathogenicity and in cultural reaction. In other words, there appear to be a great number of species, varieties, and forms, from which one might assume that the bacteria are not very stable. A condition exists here which is similar to that found in the genus Fusarium of the fungi. A discussion of the variability of the group is scarcely pertinent at this time, but it is interesting to note that the green-fluorescent bean pathogenes illustrate the point rather well. *Phyt. medicaginis* var. *phaseolicola* and *Phyt. vignae* var. *leguminophila* might have been given specific names, and no doubt would have been so treated by many investigators; but they exhibit such a close relationship to *Phyt. medicaginis* and *Phyt. vignae*, respectively, that it was considered best to show this in their name. In discussing the variability of the group, it should be pointed out also that the New York strain of *Phyt. vignae* var. *leguminophila* differs slightly from the New Jersey strain, and the English strain of *Phyt. viridiflava* is not identical with the Swiss strain, when pathogenicity is considered.

Differences in loss of virulence of the pathogene in pure culture

One point of difference among these bacteria which should be touched upon here, although it can scarcely come under the head of cultural reactions, is the variability in the loss of virulence, and even loss of pathogenicity, which exist among them. This fact has been mentioned in the individual descriptions, but it should be emphasized here where the peculiarities of each form are under discussion. The ability of the organisms to retain their virulence in pure culture varies with the species. *Phyt. flaccumfaciens* is an outstanding example of a pathogene that does not lose its virulence. Strains of this organism four and five years old are at hand which appear to have lost nothing of their ability to infect their host, or at least such a loss is not evident. *Phyt. medicaginis* var. *phaseolicola* is a second organism which may be placed in this class. One strain has been under observation for four years and still has the ability to kill young Red Kidney bean plants when these are inoculated. Certain symptoms, however, such as the stem lesions, are not so striking in appearance when produced by these old strains as when produced by a recently isolated strain, but the final result appears to be similar.

Strains of *Phyt. phaseoli* five and six years old will still cause definite pod spots. They do not, however, infect the vascular system and produce a wilt as rapidly as do recently isolated strains. Some old strains have apparently lost this ability, and a bean plant inoculated at a stem node with such an organism will show no generalized signs of the disease. *Phyt. viridiflava* and *Phyt. vignae* var. *leguminophila* lose their virulence very rapidly in pure culture. Continued inoculation of the bean plant with each, and reisolation of the organism, were necessary for inoculation experiments in order to have at hand a culture of the pathogene which showed any virulence. With *Phyt. viridiflava* the pathogenicity was at times even entirely lost.

In considering the loss of virulence in some of these species, one is forced to look upon this ability of organisms to produce disease as a rather delicate character, a variable one in many cases and one about which very little is known. The plant pathologist has been inclined, in naming species, to consider pathogenicity as a primary character. In this respect his position as plant pathologist has made him a little biased. The fermentation of a sugar is more stable, in many cases, than is the pathogenicity, and therefore is a more reliable character. We pay little attention to the sugar, however, and place importance on pathogenicity. It is there that our interest lies. It is possibly for this reason that many of these bacterial pathogenes have been overlooked in the past. All bacteria causing a blight in beans have been considered as one and the same. The problem, however, is more complicated than it appears on the surface, and probably the six forms discussed in this memoir are not all that attack beans. The cultural characteristics of the bacteria should be considered more carefully, and, when possible, differential media should be devised.

HOST RANGE

In the past many bacterial plant pathogenes have been described as occurring on certain hosts, as if this character definitely placed them. If sufficient cultural characteristics accompany such a report, no misunderstandings are likely to arise. If an insufficient description of the organism is given, however, synonyms will undoubtedly occur. Not only may a number of bacteria cause similar diseases in one species of plant, but the pathogenes themselves may have the ability to infect other species and genera of plants. This should be recognized, but just how far one should go in determining a host range for an organism it is difficult to state. The field is extremely large. When there is any doubt, however, concerning two or more species on different hosts, a certain amount of cross inoculation is necessary. With the bacterial pathogenes attacking beans, this is especially true. Here we have several green-fluorescent forms occurring on the common bean (*Phaseolus vulgaris*), and it is known that a number of green-fluorescent bacteria are pathogenic to other leguminous species. It is very desirable to know the host range of each organism. With the yellow species the confusion is not so great, but among these *Phyt. phaseoli* var. *sojense* is separated from *Phyt. phaseoli* mainly on its pathogenicity for a certain host.

A host range for a pathogene is important also for other than taxonomic reasons. For control measures it is very desirable to know what other plants may harbor the organism. Susceptible plants may then be eradicated, or their planting avoided.

Present knowledge concerning the susceptibility or the immunity of fourteen legumes to the six bean pathogenes under discussion here, is summarized in table 3. The results from many hundreds of inoculations

TABLE 3. HOST RANGE (LEGUME) OF SIX BACTERIAL PATHOGENES OF THE BEAN (PHASEOLUS VULGARIS)*

	Phytomonas phaseoli	*Phytomonas phaseoli* var. *fuscans*	*Phytomonas flaccumfaciens*	*Phytomonas medicaginis* var. *phaseolicola*	*Phytomonas vignae* var. *leguminophila*	*Phytomonas viridiflava*
Phaseolus vulgaris	+	+	+	+	+	+
P. lunatus	+	+	+	+	+	+
P. coccineus	+	+	+	+	−	+
P. angularis	−	o	+	−	o	o
Vigna sinensis	−	−	+	−	+	+
V. sesquipedalis	−	−	+	−	+	−
Glycine max	−	−	+	−	+ (pod)	+ (pod)
Lupinus polyphyllus	+	−	−	−	−	−
Dolichos lablab	+	−	+	−	+ (pod)	+
Pueraria hirsuta	−	−	−	−	+	+
Vicia faba	−	−	−	−	+	−
Pisum sativum var. *arvense*	−	−	−	−	−	−
Melilotus alba	−	−	−	−	−	−
Medicago sativa	−	o	o	−	o	o

* A plus sign denotes that infection has been obtained; a minus sign, that there is no infection; and a zero sign, that no inoculation experiments have been conducted. A plus sign followed by the word "pod" means that infection has been obtained on the pod only.

were considered in compiling this table. In all cases, care was taken to make certain that a virulent culture of the pathogene was being used. Nevertheless, when infections on a certain plant were not obtained, the experiment was repeated two and three times to make certain of the results. Even then, some unknown factor necessary for infection might have been absent. However, under the conditions maintained — a temperature of 80° C., and the use of young, rapidly growing plants as the host — it is felt that the data are very close to being correct.

It may be noticed that most of the organisms listed in table 3 show a distinct difference in their ability to infect many of the species of plants considered. *Phyt. phaseoli* var. *fuscans* and *Phyt. medicaginis* var. *phaseolicola*, however, have a similar host range as far as the table shows, and *Phyt. viridiflava* and *Phyt. vignae* var. *leguminophila* appear rather closely related in this respect.

DIFFERENTIATION OF THE BACTERIAL DISEASES FROM OTHER BEAN DISEASES NOT OF BACTERIAL ORIGIN

The bacterial diseases of the bean will not, as a rule, be confused with any other bean diseases of fungous or of virus origin. There are a few pitfalls, however, which should be considered here.

The only fungous disease that causes lesions on the stems, leaves, and pods of the bean plant is the anthracnose due to *Colletotrichum lindemuthianum*. The differences in the character of the symptoms of the anthracnose and of the bacterial blights have been emphasized repeatedly in numerous publications, and need not be described here. Any one who is at all familiar with plant diseases will be able to make the distinction between this fungous disease and bacterial blights.

The mosaic symptoms on the leaves of the bean plant produced by *Phyt. medicaginis* var. *phaseolicola* have been reported earlier in this memoir, and if these are observed alone some difficulty might arise in distinguishing them from the mosaic produced by the true virus disease. In examining infected plants as a whole, however, the separation of the two diseases on the basis of the other symptoms should be made very readily.

Fusarium martii f. sp. *phaseoli* will cause a stunting of the bean plant, a yellowing and dropping of the leaves, and sometimes a slight wilt. An examination of the roots of plants so appearing, however, would leave no doubt as to the organism causing the trouble.

Seedling wilts due to the bacteria are indistinguishable macroscopically from the hereditary seedling wilt described in a previous publication (Burkholder, 1926). If the wilt is due to bacteria, the microscope will reveal the cause. Furthermore, the hereditary wilt has never been observed except on hybrid plants.

Spots on bean pods due to sun scald are often confused with spots caused by bacteria. On certain varieties of beans, especially the garden varities,

FIGURE 15. SUN SCALD ON BEAN PODS OF THE WHITE KIDNEY VARIETY

the similarity is so great that a microscope is essential in detecting the difference. However, there are certain dissimilarities between the two types of lesions which should be noted. Sun-scald spots always are found on the exposed surface of the bean pod, and they never show the water-soaked appearance that occurs with the more common bacterial diseases. In many cases, too, the sun scald appears on the pods over the seeds. These spots are somewhat sunken, and may be of any shape from rather irregular dashes to large spots more or less circular in outline which often anastomose. In color they are brown to red-brown, according to the variety of bean.

GEOGRAPHICAL DISTRIBUTION OF THE VARIOUS BACTERIAL DISEASES OF THE BEAN

The exact geographical distribution of the various bacterial diseases of the bean is difficult to determine, since it is only recently that more than one of these diseases has been recognized. In reports of plant-disease surveys, as a rule the cause of a blight of beans has been referred to *Phytomonas phaseoli*. Probably in the majority of cases this has been correct, but since there is seldom a description of symptoms of the disease accompanying such a report one can never be quite sure. Seldom if ever is an isolation of the causal organism made to determine its appearance in pure culture. In the following paragraphs the known geographical distribution of the six bacterial diseases of the bean here considered is given. The actual distribution is much more extended than that recorded here, since there have been reports of bacterial diseases of the bean in countries not mentioned in this account. From such reports, however, there would be some difficulty in determining which bacterial pathogene was involved.

More is known concerning the distribution of *Phyt. phaseoli*, the oldest recognized pathogene of the group, than for the other pathogenes. It is of widespread occurrence in the United States and Canada, and, being a seed-borne disease, probably is to be found in most bean-growing countries. Delacroix (1899) reported the disease from France shortly after Smith (1898) first described it as occurring in America. Little has been said, however, concerning the presence of this pathogene in France since Delacroix's report; and the writer was unable to find any bacterial disease in the bean fields in Normandy and those about Paris during the summers of 1924 and 1927. Welles (1922) reported the disease in the Philippines, and Picado (1924) reported a yellow bacterium, which probably was *Phyt. phaseoli*, causing a disease of beans in Costa Rica. The pathogene was isolated by the writer from leaves and pods collected in Bermuda in 1922, and from diseased pods collected in Switzerland in 1927.

Phytomonas flaccumfaciens, a less generally recognized pathogene, is

found throughout the United States, as reported by Miss Hedges (1926). It has been isolated also, by the same investigator, from seed both from France and from Germany.

Phytomonas medicaginis var. *phaseolicola* was first observed in New York by the writer (Burkholder, 1926), and recently Miss Hedges (1928) has reported it from Montana, Wyoming, and Colorado in the West, and from Florida, Georgia, and South Carolina in the South. In Europe, M. K. T. Wieringe has isolated the pathogene in Holland, and the writer has obtained it from diseased bean pods collected in Switzerland.

Phytomonas phaseoli var. *fuscans* has been found in Switzerland causing a disease of the common bean, and Miss Hedges has isolated the organism from seed imported from South America.

Phytomonas vignae var. *leguminophila* has been found only in New Jersey and in New York.

Phytomonas viridiflava was isolated from pods collected in a market at Lucerne, Switzerland, and a similar organism was found causing a leaf spot of the scarlet runner bean near Canterbury, England.

CONTROL METHODS

The same control measures have been advocated for the various bacterial diseases of the bean, since the diseases are alike in many respects and since the life histories of the pathogenes are similar, all being seed-borne diseases. In New York the blights due to *Phytomonas phaseoli* and *Phyt. medicaginis* var. *phaseolicola* are the bacterial diseases which cause economic losses, and consequently are the ones with which control measures are especially concerned. The methods applied in holding in check these two diseases, however, appear to be effective against the other bacterial diseases of the bean also. These methods of control, which have been successful when applied, are two in number: first, the use when possible of bean varieties showing some resistance to the blights; and secondly, the use of disease-free seed in all cases. Rands and Brotherton (1925) have published a paper dealing with the varietal susceptibility of many varieties of beans to *Phyt. phaseoli* and *Phyt. flaccumfaciens*; and the writer (Burkholder, 1924) has reported on similar experiments with *Phyt. phaseoli*. Observations in the field and on inoculation work in the greenhouse leads to the belief that there is a close resistance correlation among the various bacterial diseases. Consequently, the use of any of the varieties listed in the above-mentioned articles as somewhat resistant will probably eliminate much of the trouble from all of these diseases. In New York, among the field beans or those frequently referred to as the dry shell beans, the Robust Pea bean shows considerable resistance to these bacterial diseases. Also, in the fields the blights seldom cause much damage to Yellow Eye, and White Marrow is not extremely susceptible. With reasonable precautions in selecting the seed to be planted, one should have little trouble with these varieties.

The roguing of parts of fields, and the use of seed from such parts for planting in the following year, has been practiced with success with Perry Marrow. It should be remembered, however, that this procedure can be carried out with greater ease in some seasons than in others. In a season favorable to the blight, when the diseases spread rapidly, some difficulty might be encountered. Ordinarily, however, the method should be beneficial. It should be emphasized that it is only among the varieties that show some resistance that roguing should be applied. This method of control with Red Kidney or an equally susceptible variety probably would be of little avail. The diseases, especially the halo blight, spread so rapidly through a field of Red Kidney beans that it would be impossible to remove all diseased plants and have any plants left.

The use of seed from disease-free fields is being practiced in New York by many of the better growers. A field in which there are no diseased seedlings to act as a source of infection remains reasonably free from the bacterial blights, since infection from outside comes in late and has no opportunity to do much damage. When it is impossible to find disease-free fields, the use of seed from fields which show only a small percentage of infected plants probably is more desirable than the use of seed from fields where the percentage of diseased individuals is high. Nevertheless, a very small percentage of blight in one year in a field of Red Kidney beans may develop to 100 per cent in the second year. This may be the case also with other extremely susceptible varieties.

It has been found, recently, that Red Kidney beans grown in California are entirely free from the bacterial blights, and that such seed brought to New York has produced healthy fields the first year. Ultimately, under our conditions the seed becomes contaminated. The length of time for this to happen would depend, no doubt, on the care taken of the seed. If planted next to or near a field of New-York-grown seed, it probably would become infected very quickly. If isolated in a field from all other beans, it would be safe for use as seed so long as no disease appeared in the field.

The demand for California-grown Red Kidney seed in New York has become very great, and there is on the market considerable seed labeled as being from California which probably never came from there. At least much of the seed is infected with bacterial pathogenes. The bean-growing areas of California are not known to the writer, and it is possible that certain sections do not produce blight-free seed. The location and study of areas able to produce disease-free seed would be of great benefit to the bean industry.

Spraying or dusting field beans for the bacterial blights, or even for the fungous diseases, is not to be recommended under New York field conditions. There are several reasons for this. The selection and care of disease-free seed and the use of disease-resistant varieties are simple

methods of control, and, when employed with good cultural practices, are satisfactory. Spraying and dusting are cumbersome, disagreeable, and expensive processes even when they are effective, and the bacterial blights of the bean are not of sufficient enphytotic character to warrant their expense. That the expense would be considerable is certain. Numerous applications would be necessary, since the bean plant is susceptible to the blights at all periods in its life and also is a rapidly and almost continuously growing plant. This would necessitate applications from the seedling stage until the pods were mature. Furthermore, the bean-blight problem in New York is not complicated with an insect problem which frequently makes spraying or dusting necessary. One has only the disease control to consider. Again, one must take into consideration how much damage a spray machine would do to the bean plants. Such varieties as Marrow, Medium, Pea, and Yellow Eye are of indeterminate growth habits, and not only the vines, but the pods on them would be destroyed. Even cultivators cause damage to beans, especially when the plants reach the blossoming and podding stages, and it is one of the better practices in bean culture to prepare the land so thoroughly that few cultivations are necessary.

How long the pathogenes will live in old bean vines in the fields is not known. The ability of *Phyt. phaseoli* and *Phyt. flaccumfaciens* to live for many years in a dry condition in leaves and seed, and of *Phyt. phaseoli* to live for at least a year in the soil, shows that these two organisms are rather resistant to their environment. A clean-up of old vines is desirable as a general sanitary measure, and probably would reduce chances of infection where rotation of the bean crop is short.

SUMMARY

This memoir reports an investigation of the bacterial diseases of the common bean (*Phaseolus vulgaris*). Six distinct diseases are recognized, the causal organisms of which are as follows: *Phytomonas phaseoli*, *Phyt. flaccumfaciens*, and *Phyt. medicaginis* var. *phaseolicola*, which have hitherto been reported upon; and three newly described organisms, *Phyt. phaseoli* var. *fuscans*, *Phyt. vignae* var. *leguminophila*, and *Phyt. viridiflava*.

Careful and extensive inoculation experiments have proved the pathogenicity of each organism.

The symptoms of each disease on the bean plant are described in full and their similarities and differences are noted.

Various phases of the life history of the organisms are described. *Phyt. flaccumfaciens* is primarily a vascular parasite; *Phyt. phaseoli*, *Phyt. phaseoli* var. *fuscans*, and *Phyt. medicaginis* var. *phaseolicola* may or may not be vascular parasites; *Phyt. vignae* var. *leguminophila* under certain conditions may be found in the xylem; but *Phyt. viridiflava* cannot be considered a vascular parasite.

The morphology and the cultural characteristics of each pathogene are given. *Phyt. viridiflava*, *Phyt. vignae* var. *leguminophila*, and *Phyt. medicaginis* var. *phaseolicola* belong to the green-fluorescent group of bacterial plant pathogenes; *Phyt. phaseoli* and its variety *fuscans* belong to the *Campestre* group; and *Phyt. flaccumfaciens* belongs to the *Stewarti* group.

The pathogenes vary in their host range over a dozen leguminous plants which were used in a series of inoculation experiments.

Control measures for the diseases are discussed.

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The following list of articles by no means contains all the references to the bacterial diseases of the bean. It does contain the more important ones, together with certain foreign references which would help in determining the distribution of the various diseases. American literature is full of short descriptions and paragraphs mentioning bacterial affections of this crop. Such articles are disregarded here unless they have contributed something to our knowledge of the subject. Other articles and books listed, which do not have a direct bearing on the diseases under discussion, contain descriptions of methods and technic which have been used in this work.

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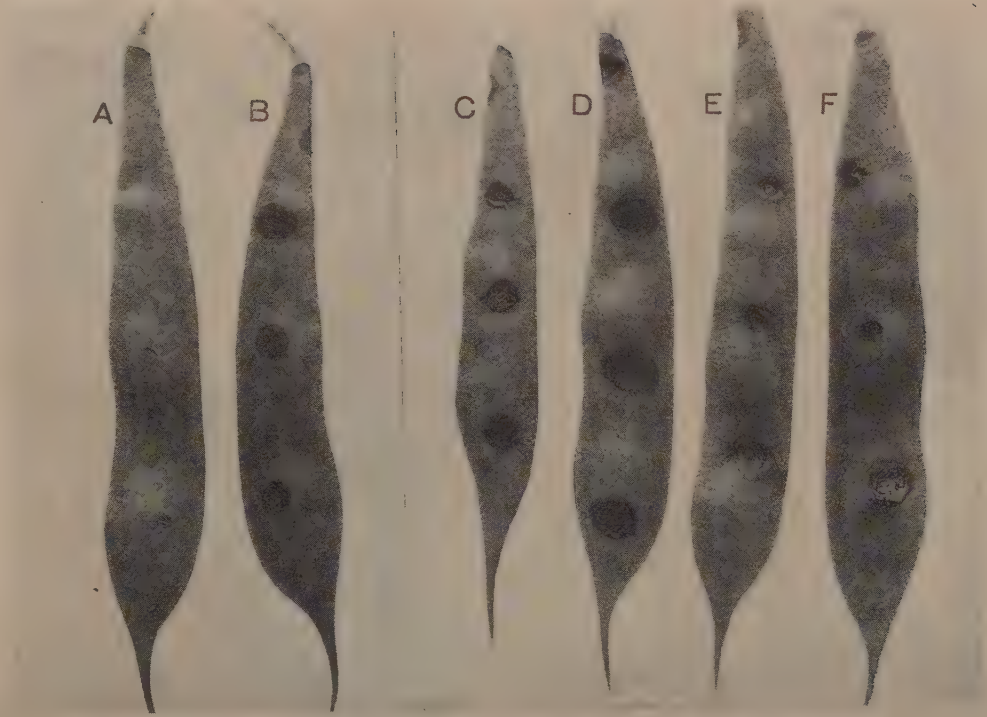
Memoir 125, *Inheritance and Linkage Relations of Virescent Seedlings in Maize*, the second preceding number in this series of publications, was mailed on January 23, 1930.

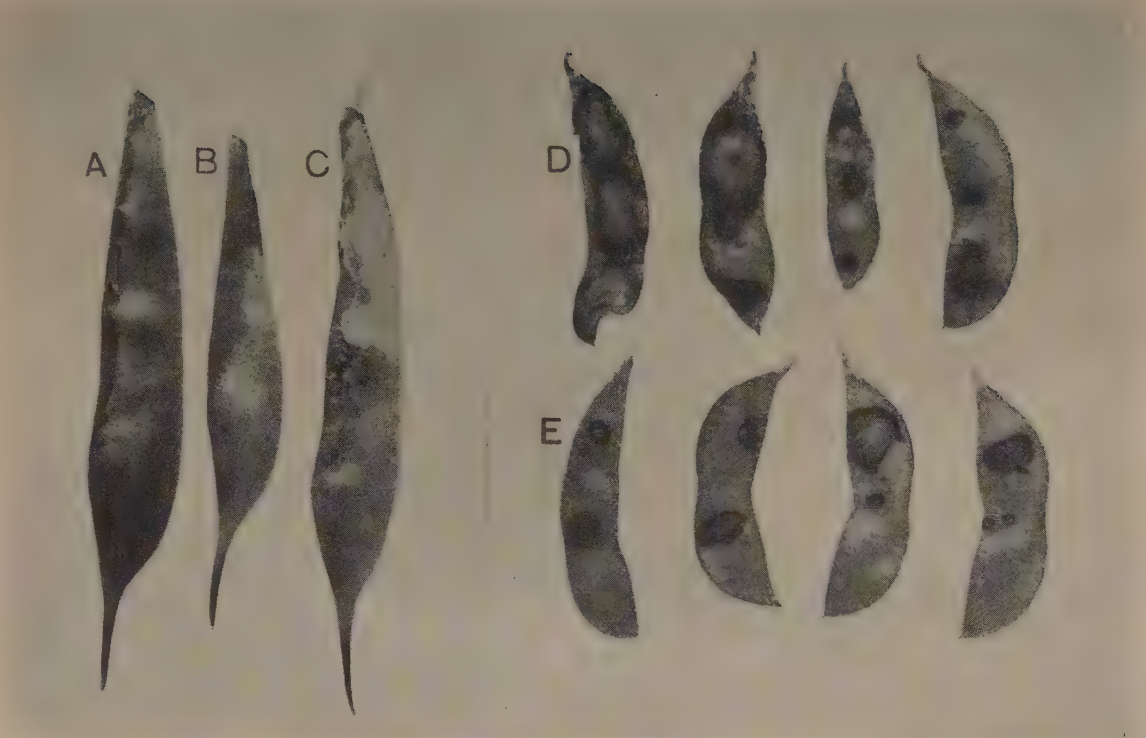
Memoir 126, *Effect of Storage in Finely Divided Feeds upon the Stability of the D Vitamin of Cod-Liver Oil*, was mailed on January 30, 1930.

LEAF OF A GARDEN VARIETY OF BEANS, WITH SMALL, SLIGHTLY RAISED LESIONS CAUSED BY PHYTOMONAS PHASEOLI, AND PODS FROM THE SAME PLANT



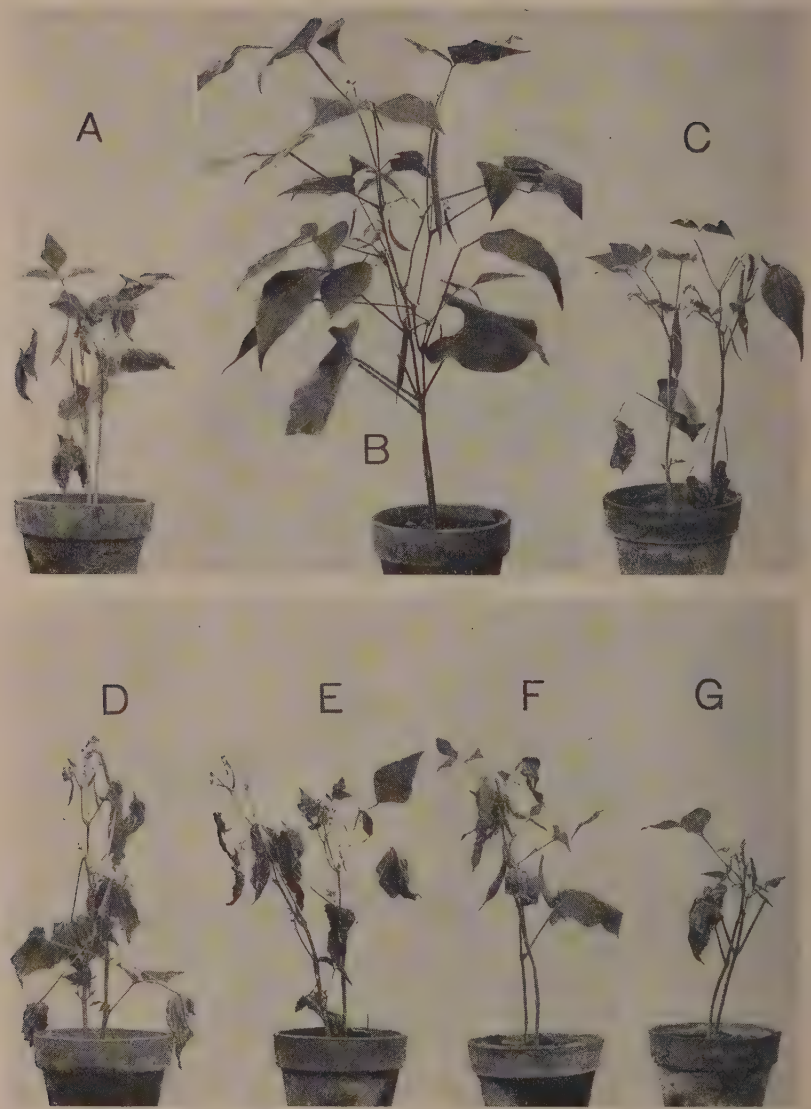
RED KIDNEY BEAN PODS SEVEN DAYS AFTER INOCULATION WITH BACTERIAL PATHOGENES

The pods were injured at the time of inoculation. A, *Phytomonas flaccumfaciens*; B, *Phyt. medicaginis* var. *phaseolicola*; C, *Phyt. vignae* var. *leguminophila* (New York strain); D, *Phyt. viridiflava* (Swiss strain); E, *Phyt. vignae* var. *lejuminophila* (New Jersey strain); F, *Phyt. viridiflava* (English strain)



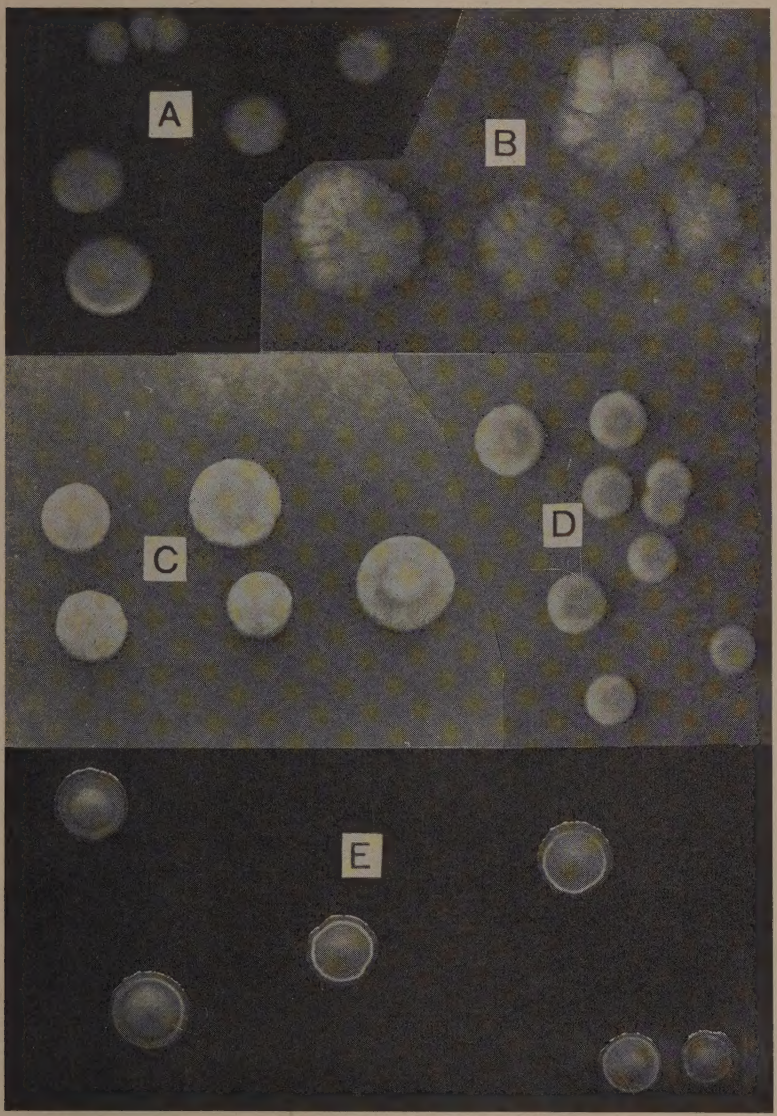
A TO C, SUTURE LESIONS ON RED KIDNEY BEAN PODS DUE TO INOCULATING THE PEDICEL WITH BACTERIAL PATHOGENES
D AND E, SOYBEAN PODS SHOWING LESIONS DUE TO ARTIFICIAL INOCULATIONS WITH BACTERIAL PATHOGENES

A, *Phytomonas medicaginis* var. *phaseolicola*; B, *Phyt. phaseoli*; C, *Phyt. phaseoli* var. *fuscans* (this type of lesion is often caused also by *Phyt. flaccumfaciens*); D, *Phyt. vignae* var. *leguminophila*; E, *Phyt. viridiflava*



WILTS OF RED KIDNEY BEAN PLANTS ONE MONTH AFTER INOCULATION WITH BACTERIAL PATHOGENES

The plants were inoculated at the first leaf node. A, *Phytomonas medicaginis* var. *phaseolicola*; B, check; C, *Phyt. phaseoli* var. *fuscans*; D and E, two strains of *Phyt. phaseoli*; F and G, two strains of *Phyt. flaccumfaciens*



SURFACE COLONIES OF VARIOUS BACTERIAL PATHOGENES OF THE BEAN

A, *Phytomonas vignae* var. *leguminophila*; B, *Phyt. viridiflava*; C, *Phyt. phaseoli* var. *fuscans*; D, *Phyt. flaccumfaciens*; E, *Phyt. medicaginis* var. *phaseolicola*

A to D, Colonies seven days old, temperature 27° C., beef-extract agar (pH 7.0), × 2. E, Same conditions except that colonies are fourteen days old and agar is slightly acid